SHOUT
SHARE
IT

Cand.
3/11/17

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

Editorial Staff

E. G. Conklin—*Princeton University.*
Jacques Loeb—*The Rockefeller Institute for Medical Research.*
George T. Moore—*The Missouri Botanical Garden.*
T. H. Morgan—*Columbia University.*
W. M. Wheeler—*Harvard University.*
E. B. Wilson—*Columbia University.*

Managing Editor

Frank R. Lillie—*The University of Chicago.*

Volume XXX.

WOODS HOLE, MASS.
JANUARY TO JUNE 1916

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

10931

CONTENTS OF VOLUME XXX.

No. 1. January, 1916.

PAGE.

Wodsedalek, J. E. *Causes of Sterility in the Mule* 1

Kanda, Sakyo. *Studies on the Geotropism of the Marine Snail, Littorina littorea* 57

Kanda, Sakyo. *The Geotropism of Freshwater Snails* 85

No. 2. February, 1916.

Lewis, Margaret R., and Robertson, Wm. Rees B. *The Mitochondria and Other Structures Observed by the Tissue Culture Method in the Male Germ Cells of Chorthippus curtipennis Scudd* 99

Robbins, W. J. *Notes on the Physiology of Fucus spermatozoids*. 125

Moore, A. R., and Kellogg, F. M. *Note on the Galvanotropic Response of the Earthworm* 131

Wilder, Harris H. *Palm and Sole Studies.* I to IV 135

Newman, H. H. *Heredity and Organic Symmetry in Armadillo Quadruplets* 173

No. 3. March, 1916.

Wilder, Harris H. *Palm and Sole Studies.* V and VI 211

No. 4. April, 1916.

Willis, H. S. *The Influence of the Nucleus on the Behavior of Amœba* 253

Sumner, F. B. *Notes on Superfetation and Deferred Fertilization Among Mice* 271

Goodale, H. D. *Further Developments in Ovariotomized Fowl* . . 286

Bachhuber, L. J. *The Behavior of the Accessory Chromosomes and of the Chromatoid Body in the Spermatogenesis of the Rabbit* 294

No. 5. May, 1916.

Lillie, Ralph S. *The Theory of Anæsthesia* 311
Glaser, R. W., and Chopman, J. W. *The Nature of the Polyhedral Bodies Found in Insects* 367
Child, C. M. *Axial Susceptibility Gradients in the Early Development of the Sea Urchin* 391

No. 6. June, 1916.

Eighteenth Annual Report of the Marine Biological Laboratory . . . 407
Macklin, C. C. *Amitosis in Cells Growing in Vitro* 445

Vol. XXX. *January, 1916.* *No. 1*

BIOLOGICAL BULLETIN

CAUSES OF STERILITY IN THE MULE.[1]

J. E. WODSEDALEK.

(NINE PLATES.)

CONTENTS.

I. Introduction 1
II. Comparison of the Horse and the Ass 3
III. Characteristics of the Mule 7
IV. Material and Methods 9
V. Appearance of the Testicular Tissue 9
VI. Spermatogonia 10
VII. Primary Spermatocytes 15
  1. Early Prophase 15
  2. Synapsis 17
  3. Abnormalities in Mitosis 23
VIII. Giant Cells 32
IX. Multinucleate Cells 33
X. Destruction of the Germ Cells 34
XI. Summary 36

INTRODUCTION.

For several years the author has been contemplating to make a study of the germ cells of the mule with the aim of offering a thorough explanation of the causes of sterility in this hybrid, but it was not until the past year that material for such an investigation was available. Cytological studies of the sex cells, especially those of animals, in recent years have added much to our knowledge of the mechanism of heredity; and such studies have kept fairly good pace with the comparatively rapid advances in our knowledge of plant and animal breeding which have been made since the rediscovery in 1900 of the results of Mendel. And while this study is now recognized as one of the chief methods of confronting many problems in genetics, and must be carried on in correlation with animal and plant breeding before any adequate explanation of the results of hybridization can be obtained, the study of the germ cells in hybrids themselves has thus far been somewhat neglected.

---

[1] From the zoölogical laboratories, University of Idaho.

Guyer ('00) in his excellent work on the spermatogenesis of normal and hybrid pigeons says: "It is a remarkable fact that no attempt has been made so far to investigate carefully the spermatogenesis or ovogenesis of hybrid forms. In all the mass of literature discussing or touching upon hybridism, so far as I have been able to ascertain, there has been in no instance an approach to a thorough study of the germ cells. Yet almost every writer states that through the study of hybrids, we have perhaps the best opportunity for gaining a clew to many of the most vital points in the great problem of heredity. A number of investigators have remarked that in certain instances the anthers, ovary, or testes as the case might be, were defective, and have let the matter go at that."

It is even more remarkable now, since fifteen years have elapsed and no further attempt has been made to investigate carefully the spermatogenesis or ovogenesis of other hybrid forms. Professor Guyer's work on hybrid pigeons which was published in 1900, the same year in which Mendel's investigations were rediscovered, so far remains as the only outstanding piece of work on that particular subject. It is all the more remarkable when we consider the fact that such a great deal of cytological work has been done during the past fifteen years and that by far the largest bulk of the work on hybridization has been done during the same period.

The mule is probably one of the best known hybrids for he is raised in practically all parts of the civilized world. He has been known for many centuries and has been used more or less in Europe since the days before Christ, for Varro who wrote in the first century B.C. refers to mules in Roman agriculture. Sterility in mules has no doubt been a subject of discussion for as many centuries but the real nature of the cause of sterility in these hybrids has heretofore never been carefully investigated. The spermatogenesis of the horse which was carefully worked out at the University of Wisconsin Zoölogical Laboratories a year ago (Wodsedalek, '14), has rendered several phases of this difficult problem much more intelligible than would have been possible otherwise. It might be added that certain phases of this problem would have become even more perspicuous had the spermatogenesis of the ass also been carefully worked out and thoroughly understood; but unfortunately the jack material has so far been unavailable.

Comparison of the Horse and the Ass.

Hayes ('04) says: "Owing to the extreme want of uniformity in the gaps left, during the process of evolution, between descendants from similar ancestors, we are unable to lay down any exact general rules for classification. The inclusion of horses, asses, and zebras in the genus *Equus* admits of no controversy, because they are the only possessors of the distinguishing characteristic of having only one complete hoofed toe on each foot."

While there seems to be no doubt that the horse, ass, and zebra have descended from common ancestors, at the present time, there is considerable difference between the horse and the other two Equidæ. The only distinctive difference between the asses and zebras in general seems to lie in the tiger-like stripes which are invariably lacking in the ass. The differences between asses and horses are marked, as can be seen from the following quotation of a detailed comparison by Hayes ('04). "Some of the following differences between asses and horses are relative, and others absolute. Most of these differences also exist between zebras and horses.

"1. The ass has, practically speaking, chestnuts only on the forelegs, which is a peculiarity that is met with in certain breeds of horses (p. 319). In some cases, the ass has vestiges of chestnuts on his hind legs. The chestnuts and ergots (p. 319) of the ass are much thinner than those of the horse.

"2. The ass has a tufted tail, somewhat like that of an ox, an erect mane, and no forelock. The horse, with the exception of Prjevalsky wild horse (p. 640), has a bushy tail, drooping mane, and a forelock, when they have been allowed to grow. The difference in the mane is due to the length of the hairs of the part. In almost all breeds of horses the hairs of the tail grow long from the root of the dock. In the ass they do so only as they approach the end of the dock.

"3. As a rule the ass has five loin vertebræ, and the horse six (p. 422). I have never heard of an instance, in the domestic ass, of the number of these bones exceeding five. If, however, we examine the skeleton of the Mountain Zebra which is in the museum of the R. C. S. Lincoln's Inn Fields, we shall see that it has six loin vertebræ. The number of these bones is subject to variation in all vertebrates.

"4. In the horse, the lachrymal duct, which is the canal that conveys tears from the eye on each respective side into the nostril, has its opening near the inferior commissure of the nostril, and on the line of union between the dark-colored skin and the pink mucous membrane. In the ass and mule, it is situated at the inner face of the outer wing of the nostril. This orifice is sometimes double.

"5. In the ass, the false nostril extends higher up than in the horse.

"6. The male ass has two rudimental teats in the form of small tubercles. They are usually absent in the horse.

"7. The vocal sounds of the ass (braying) are produced in a different manner from those of the horse (neighing). . . . We may, therefore, conclude that braying can be performed only during strong contractions of the muscles of the abdomen and chest. It is evident that this muscular contraction is not required in the neighing of the horse.

"8. In the ass, the deep depression at the base of the epiglottis is covered by a thin membrane, which is capable of vibrating, and which is wanting in the horse. It may have some influence in causing the voice of the ass to differ from that of the horse. . . .

"9. The ass hardly ever has any irregular markings on its coat such as a 'star,' 'blaze,' 'reach,' or 'stockings,' all of which are very frequent among horses. A small star, on one or two occasions, is the only mark of the kind I have ever seen in the ass, of which animal I have not had much experience.

"10. I believe I am correct in saying that the color of the ass is never of a bright bay, chestnut, red or blue roan, or nutmeg gray. I have seen mules of an iron-gray color; but have not observed it in the ass. This conservatism in color and freedom from irregular markings, shown by the ass, is very remarkable; considering how greatly the coat of the horse varies in this respect and that the ass has, in all probability, been longer under the influence of domestication than the horse.

"11. The ass is higher over the croup, than at the withers which is a peculiarity that tends to make his withers appear unduly low (p. 241). The spines of the vertebræ at the withers are only a little shorter in the ass than they are in the horse. As a rule horses are higher at the withers than they are at the croup.

"12. The horse's dock is thicker, stronger, and shorter than that of the ass.

"13. The horse, on each side of his croup and covering his pelvis, has, underneath, and closely adhering to the skin of the part, a thick and extremely dense layer of connective tissue, which is so close and hard, that it looks like horn, when the skin has been tanned and dried. These two patches of thickened skin are separated from each other about four or five inches apart, so that there is a strip of skin of ordinary thickness running down the croup towards the tail. . . . The 'shell' is connected to the skin so closely that the two form one piece; although their respective consistencies are different. If a section be made through the hide their line of union may be readily seen. In the ass, the 'shell' is not confined to the skin that covers the pelvis; but also extends over the ribs, which are consequently not as sensitive to the effects of blows as are those of the horse. . . .

"14. The ass has no tufts of hair at the fetlocks (p. 290).

"15. Messrs. Tegetmeier and Sutherland appear to have been the first to note the difference between the respective periods of gestation of asses and horses; the former period being twelve months; the latter, eleven months.

"16. The foot of the horse is more highly specialized than that of the ass. One of the best marked evolutionary changes in the equine foot, was the gradual curtailment of its posterior bearing surface (frog and sole), until, in the horse (Fig. 382), the length of this bearing surface is not much greater than its width, and is included between the heels and the 'toe' of the hoof. In the ass, this bearing surface is relatively longer than in the horse, and extends some distance beyond the heels to the rear (Fig. 443). Also, the shape of the foot of the ass, from above downwards, is more or less cylindrical; and that of the horse is more or less in the form of a truncated cone.

"17. The teeth of the horse are more highly specialized than those of the ass. . . . We can see in Fig. 643, that the gradual lengthening of the crowns of the molar teeth is a well-marked feature in equine evolution; and from a study of the mechanism of equine dentition, we learn, that for purposes of food-prehension and mastication, the growth of the incisors must be proportionate to that of the molars. Hence, we may assume that the crown of the incisors, like those of the molars, have gradually lengthened during the evolution of the horse; and consequently that the acuteness of the angle made by the upper and lower incisors of the horse of today increases with age, to a greater extent than it did in the case of his ancestors. On turning to the domestic ass, we find that in old donkeys (Figs. 448, 449 and 450), the angle in question is greater in the horses of similar ages (Figs. 445, 446, and 447). Consequently we may infer that the teeth of the domestic ass are of an older type than those of the horse. Also, the incisors of the donkey are relatively narrower than those of the horse." In addition it might be said that the ears of the ass are much larger and longer.

All of the differences enumerated above are skin-deep, or anatomical in nature. The present study has revealed another difference, a cytological one, which is probably the most important, for it lies in the finer structure of the cells of these animals and no doubt is at the base of all of the differences perceptible to the naked eye. It was learned that the cells of the horse contain thirty-seven chromosomes (Wodsedalek, '14). The present study shows that the cells of the mule possess fifty-one chromosomes. This would suggest that the cells of the ass possess about sixty-five chromosomes, if nothing unusual happens in the development of the hybrid, thus making the remarkable difference of twenty-eight chromosomes between the cells of the horse and those of the ass. This matter is considered at length in the latter part of the paper.

Characteristics of the Mule.

The mule is a hybrid having for sire a jackass or male ass, commonly termed a jack, and a mare for dam. If, however, a stallion be bred to a she-ass which in the United States is called a "jennet" and in England a jenny, the result is a hybrid known as a hinny. It seems to be a well-established fact that no special distinction with respect to appearance or conformation can be made between mules and hinnies, because in both of these hybrids of both sexes the proportional resemblance to the horse and ass is of infinite variety. Mares are generally taller than jennets, hence the mules as a rule are of a greater height than hinnies, which appears to be the only difference between these two hybrids.

There seems to be a great deal of difference of opinion as to which one of its parents the mule most resembles. For example, Hayes ('04) says: "The coat, mane, tail, feet, and voice are more or less intermediate between those of the horse and those of the ass. The hind chestnuts in some cases are well developed, as in the ordinary horse; in others, they are in a comparatively vestigial form, and one or both may be absent. These hybrids "resemble the ass more than the horse in their placid temper, great adaptability to work, and longevity. In carrying or drawing loads they are superior to horses of the same weight, and they can obtain the necessary energy from food which horses cannot digest, as for instance, the reeds on which the mules of the south of France live. I have experimentally established the fact that their digestive power is higher than that of horses" (Sanson).

Lydekker ('12) says; "In the case of both mules and hinnies the general build and appearance of the animal accord with the type of the sire, although in the manner of bodily size the dam is followed. Mules are therefore asinine in appearance, although with a more horse-like tail, and relatively large ears; whereas, the more horse-like hinny is small. If, however, females of the great Poitou ass were to be utilized for hinny-breeding, the progeny would probably be of larger stature. One exception to the ass-like character of the mule is that it lacks the white belly of its male parent."

Wilcox ('07) says: "It is not true, however, as sometimes asserted, that the mule eats less than the horse. On the contrary the mule has an excellent appetite. However, the mule can go longer without food and can live on coarser and more unpalatable food than could be expected to give results with the horse. Contrary to a prevailing belief also, the mule is equally susceptible to various diseases as the horse. In the Philippines our mules suffered as much as horses from surra. Glanders has always been dreaded as one of the scourges of the army mule. Even colic and other digestive troubles familiar to horse raisers also occur among mules."

Plumb ('06) says: "The characteristics of the mule partake of both sire and dam. There is the long ear, slender body, tufted or slightly haired tail, and small, slender foot, and braying voice of the ass."

"The temperament of the mule is quiet and patient, while for steadiness under the collar and hard pulling he has no equal in the equine world. . . . Horses are more nervous and uncertain in temperament than mules, and are more subject to fright and consequent runaway.

"The endurance of the mule is remarkable. . . . Mules usually live to a greater age than horses, and perform their work with regularity and on less feed, a most important point in their favor. Cases are recorded of mules living to seventy years of age. . . . The resistance of the mule to disease, its activity, sureness of foot, docility, and easiness of keep, have resulted in its finding much favor in the army service." The sureness of foot is no doubt inherited from the ass.

That mules and hinnies are sterile is a well-known fact. And if degeneration of the sex cells of all of these hybrids is as pronounced as it was found to be in the material studied in this investigation—and in all probability it is—the cause of sterility will remain obvious. A few cases of fertile mules have been recorded, but careful investigations show that such records are not authentic and practically all of the writers on mules regard them as unreliable.

Lydekker ('12) says: "With the possible exception of a few instances in which the female is stated to have produced offspring, the mule is sterile; as, indeed, might be expected to be the case when the difference between its parents is borne in mind."

Hayes ('04) in remarking about the matter of sterility of mules says: "Scientific research has amply proved that mules and hinnies are absolutely sterile, although cases are on record of induced lactation occurring in females of this kind. . . . Accounts not infrequently appear in the American and other papers, of mules which are seen suckling young, and the conclusion is at once arrived at that these young are the offspring of the animals that are supporting them, but it may be regarded as perfectly certain that they are merely adopted foals, which by their endeavors to suck female mules have developed in the latter abnormal lactation."

Material and Methods.

The material for this study was obtained through the courtesy of my friend, Dr. J. W. Kalkus, professor of histology and pathology at the State College of Washington, Pullman. Dr. Kalkus made every possible effort to secure the material for my studies and generously placed at my disposal material obtained from three animals.

The hybrids were about two years old at the time the material was removed from them. It is probable that all three of the mules were by the same sire. The tissue was fixed in Flemming's fluid, and stained with Heidenhain's iron hematoxylin. The sections were cut from five to nine microns thick.

Appearance of the Testicular Tissue.

The first noticeable difference in the testicular structure of the mule and the horse is that the seminiferous tubules of the mule are in general much smaller than those of the horse. There is considerable variation among the tubules of the mule. The diameter of some tubules is fully twice that of others, and all gradations between the two extremes may be found. The large tubules are characterized by large clear areas in their lumens. This clear space diminishes as a rule in proportion to the diameter of the tubules. In most of the small tubules the cells occupy all of the lumen. This structure, however, is not always the case in the respective tubules, for occasionally we find large tubules with solid lumens and on the other hand small tubules with clear lumens.

The difference in the interstitial cells is quite obvious. They are much larger, much more numerous, and more regularly distributed in the horse than they are in the hybrid. The nuclei in the former are large and usually contain one or two large nucleoli, and several small karyosomes; while those of the mule are small and contain many small karyosomes, and the large nucleolus is not as constant as it is in the horse. The cytoplasmic content of these cells in the horse, too, is larger and not as variable in size as in the mule.

SPERMATOGONIA.

The spermatogonial cells of the mule are considerably larger than those of the horse in the corresponding stages. They generally occupy the usual position next to the tubule wall, though occasionally they are crowded out of this position by the numerous nurse cells and pushed further in toward the lumen. They occur in relatively fewer numbers in the hybrid than they do in the horse. In cross sections of the tubules they very seldom form a complete ring within the tubule wall, and are never found in a crowded condition neither along the entire tubule wall nor in small areas, as is often the case in normal mammalian tissue. The cells are usually far apart, and it seems safe to state that in a large majority of the tubules only about twenty to thirty per cent. of their inner surfaces is covered with these cells. It seems equally as correct to say that in cases where the spermatogonial cells are most abundant they never exceed covering more than sixty per cent. of the tubule's inner surface; and it might be said that such extreme cases are rarely found. Many of the tubules contain only a comparatively few spermatogonial cells and not infrequently tubules are found in which no cells can be identified as such. The cells occupying the position usually occupied by the spermatogonia greatly resemble the ordinary nurse cells.

During the prophase of the spermatogonial cell the nucleus is round and contains a large nucleolus which is usually heart-shaped, several small karyosomes, a large number of small granular masses and extremely thin linen strands (Figs. 1 and 2). In this respect the cells resemble those of the horse in the corresponding stages, except that they are much larger. No centrosome can be detected at this stage although a dense mass of cytoplasm can often be seen near the nucleus. This is in all probability the idiozome. These cells have definite walls which mark them off clearly from the neighboring cells. They are almost spherical and have the appearance of smear cells, a condition presumably due to the fact that they are not crowded.

As growth proceeds all parts of the cell increase in size. When the maximum size is reached, dense masses of chromatin make their appearance which become more and more defined until the chromosomes of various shapes and sizes are formed. Sometimes the chromosomes are so close together that an accurate count is impossible; but more frequently they are sufficiently separated so that many definite counts were possible.

There are fifty-one chromosomes found in the spermatogonia of the mule. Fifty of these are the ordinary chromosomes or autosomes, and one is the accessory which is larger than the others. Fifty-one chromosomes is fourteen in excess of the number found in the spermatogonial cells of the horse which has, at least in some breeds, a total of thirty-seven, thirty-six autosomes and one accessory (Wodsedalek, '14).

A considerable amount of time was spent in an attempt to ascertain which of the chromosomes are of paternal and which of maternal origin, or in other words which were contributed by the jack and mare respectively. And while the author has obtained some fairly definite ideas in regard to the individuality of the chromosomes in the mule, he does not feel that any positive statements in regard to the matter can be made until the sex cells of the ass will have been thoroughly investigated.

It is quite certain, however, that the accessory chromosome retains its individuality, for its appearance in the mule is identical to that of the accessory of the horse. The mule no doubt received the accessory from the mare in the same manner as it is contributed by the dam to her male offspring in the horse, in view of our knowledge of the behavior of this body in the spermatogenesis of the horse (Wodsedalek, '14), and particularly according to our acknowledge of the behavior of the accessory chromosomes in relation to sex determination in another mammal, the pig (Wodsedalek, '13). In regard to the other chromosomes it is definitely known that in the horse they vary considerably in size and shape (Wodsedalek, '14), and the same is probably the case among the chromosomes of the ass. And the fact that some of the chromosomes of the ass are apparently of the same size or about the same size as some of the chromosomes of the horse, makes it rather difficult to discriminate all of the maternal from the paternal chromosomes in the hybrid. It appears, however, that most of the large chromosomes in the mule are paternal in origin. A careful study of the spermatogenesis of the ass should throw considerable light on this subject.

It is also definitely known, as was stated before, that the horse, at least of some breed or breeds, possesses thirty-six chromosomes besides the accessory, or sex-determining chromosome, in the spermatogonial cells. The reduced number of the ordinary chromosomes in this animal is eighteen, which number enter into the formation of the one type of sperm, and eighteen plus the accessory or nineteen in the other type. Since the spermatogenesis of the horse with regard to the accessory (Wodsedalek, '14) bears such a striking resemblance to the spermatogenesis of the pig, in which animal the problem of sex-determination was carefully worked out (Wodsedalek, '13), the author in his paper on the spermatogenesis of the horse assumed that the sperm cells possessing eighteen chromosomes are male determining and those possessing nineteen are female determining. It was further assumed on account of the behavior of the accessory in the horse that the oogonia of the dam contain thirty-eight chromosomes, or thirty-six autosomes and two accessories, while the mature ova contain the reduced number of nineteen chromosomes, or eighteen autosomes plus one accessory.

The above figures are in all probability correct, but the actual count of the chromosomes in the female tissue must be made before it can be regarded as authentic, regardless of the strong evidence obtained in favor of the assumption through the comparison of the behavior of the chromosomes in both the male and female germinal and somatic cells of the pig. Great as the similarity is, the mere fact that the pig is the only case among the vertebrates in which the relation of the accessory chromosomes to sex-determination has been clearly and completely demonstrated, any remarks based on such a comparison must necessarily be of a more or less reserved nature.

The investigations on the relation of the accessory chromosome, or group of chromosomes, to sex-determination in at least another vertebrate, the man, must not be overlooked. And while there is some difference of opinion in regard to this matter in man and the results are not entirely conclusive, they should nevertheless add more strength to the above assumption. Guyer ('10) found that the spermatogonia of man contain twenty-four chromosomes; and that twelve chromosomes appear for division in the primary spermatocyte of which ten are evidently bivalent and two accessories. During division ten chromosomes pass to one pole and ten plus the two accessories to the other, giving rise to two different types of secondary spermatocytes which eventually give rise to two types of spermatozoa; the one type containing ten chromosomes and the other ten plus the two accessories.

Montgomery ('12) confirms the number of chromosomes found by Guyer but disagrees with him in regard to the accessory. Von Winiwarter found forty-seven chromosomes in the male, of which forty-six unite at reduction and form twenty-three bivalents and the accessory remains unpaired. Two types of spermatozoa are produced, the one containing twenty-three chromosomes and the other twenty-three plus the accessory, or twenty-four chromosomes. In the female he had some difficulty in obtaining the exact number of chromosomes, but his best counts gave forty-eight, which number fits in with the results obtained in the male.

The cause of the difference of opinion is probably due to the fact that Guyer used Negro material in his investigation, while Von Winiwarter studied tissue obtained from a white man. In view of the difference between these two human races it is not at all improbable that a difference in the number of chromosomes also exists. The Negro is fully as far removed from the white man as is the ass from the horse, where a great difference in the number of chromosomes apparently exists. This vast difference in number appears to be, at least in part, responsible for the sterility of the mule. The difference between the white man and the Negro in regard to the number of chromosomes according to Guyer and Von Winiwarter is equally as great, but unfortunately the mulatto is fertile.

The number of chromosomes in the spermatogonial metaphase stage of the mule is, as was stated before, fifty-one, or fifty autosomes and one accessory. The number in the corresponding stage of the horse is thirty-seven or thirty-six autosomes and one accessory; while the reduced number is eighteen in the one type of sperm and nineteen, including the accessory, in the other. According to the previous discussion it seems fairly safe to predict that in the oögonial cells of the mare there are thirty-eight chromosomes or thirty-six ordinary chromosomes and two accessories, and that the reduced number in the matura ova is nineteen including the one accessory. This would suggest that nineteen of the fifty-one chromosomes including the accessory are maternal in origin or contributed by the dam, and the remainder or thirty-two are paternal in origin or contributed by the jack. On the customary inference that the reduced number of chromosomes is always one half the number in the spermatogonial and somatic cells we should expect to find sixty-four chromosomes in the ass, with the possible addition of one accessory in the jack and two in the jennet; thus making a total of sixty-five and sixty-six respectively in the two sexes of this animal.

These plausible figures for the jack and jennet would, of course, be expected only in the event that no cellular abnormalities occurred in the mule from the time of the fertilization of the dam's ovum by the jack's sperm, through the succeeding developmental stages of the hybrid up to maturity. In such a case the full quota of chromosomes would be handed on to the spermatogonial cells of the mule.

The normal mitotic figures of the spermatogonial cells seem to indicate that probably no such abnormalities occur in the various preceding stages. But even such an apparently normal condition cannot be relied upon too strongly. For while the spermatogonial cells are normal during mitosis, as near as can be detected, that does not necessarily preclude that nothing unusual happens in the developmental stages of the hybrid, particularly at the time of fertilization and early cleavage stages. At any rate it would not be at all surprising if something unusual did happen to bring about the fifty-one chromosomes. The need of a careful study of the chromosomes in the jack is obvious. A study of the sex cells in both sexes of the hinney, as well as those of the female mule and she-ass or jennet, would also add materially to this immensely interesting problem of sterility of the horse and ass hybrids.

Fig. 3 represents a polar view of the metaphase stage of a spermatogonial cell. The fifty-one chromosomes are distributed throughout the entire plane of the equator and the larger ones, as a rule, are arranged along the edge. The heart-shaped accessory can be readily distinguished not only on account of its shape, but also on account of its large size. It is invariably at or near the edge of the equator. Fig. 4 is a side view of the spindle and a representation of one of the many perfect spindles found in this stage. There are no scattered chromosomes to be seen; each enters the equatorial plate and divides. The fact is further evidenced by the perfect divisions which follow. During the anaphase stage the chromosomes move to the opposite poles. Figs. 5 and 6 show that there are no stragglers among the chromosomes. The accessory which also divides in this stage, can practically in all cases, be detected on account of its larger size. In the telophase stage when the chromosomes are at the opposite poles the cytoplasm begins to constrict in the center. Soon the chromosomes become ragged and later disintegrate while the nuclear membrane makes its appearance. Following the last spermatogonial division the cell enters into the period of great growth and represents the early stage of the primary spermatocyte.

Primary Spermatocytes.

1. *Early Prophase.*

The most important phase of this problem lies in the primary spermatocytes. These cells are the result of the last spermatogonial division as in normal forms, and while their behavior is always interesting and more or less difficult to analyze in any case of spermatogenesis, it is especially so in this hybrid. From the time of the appearance of these cells until the period of their final destruction one is confronted with numerous fascinating categories.

During the early prophase the primary spermatocytes resemble the early spermatogonial stages, except that the cells are larger and the linin strands a little more conspicuous. The large nucleolus is again very distinct and appears as it did in the spermatogonia. A large, irregularly shaped karyosome is also usually present, as are a number of small ones. As the cell increases in size the linin strands become more conspicuous and the chromatin granules more numerous. The karyosomes, too, become larger and are surrounded by dense masses of chromatin granules. One of the karyosomes is always large and has from eight to twelve linin strands radiating from it. Frequently a few strands can be seen radiating from the small karyosomes. These radiating strands together with many others, form a continuous network of linin. Later the chromatin granules begin to mass around the linin strands and the karyosome begin to disappear (Fig. 7). Soon all of the chromatin material seems to be arranged along the linin strands, which take on the appearance of a network of chromatin threads. The threads are very granular and vary a great deal in length and somewhat in thickness (Fig. 7).

There is no definite arrangement of these threads in the network of the various cells. Each cell seems to have its own hit-and-miss tangle. In some cells the long threads are more numerous and the short connecting threads are proportionately fewer in number. In others there are many short components forming the network. The nuclei at this stage vary considerably in size.

Synizesis, which is so commonly observed in the horse tissue, does not occur in the mule. The nearest approach to it is a small clump of threads which is frequently seen and seems to be formed in the position occupied by the large karyosome. This clump persists in many cells until the threads break up into chromosomes. The mass of chromosomes resulting from such a clump of threads can also be frequently seen. It is probable that the clump of threads is brought about by the tangling up of the linin strands, which extend from the karyosome.

The spireme stage which follows synizesis and synapsis in the horse is also lacking in the mule. The expanded network takes its place, and only parts of the network resemble the spireme of the horse. Even in cases where long threads occur the resemblance is slight, for invariably the thread is made up of portions of various thicknesses, while the spireme in the horse is of a more uniform diameter.

2. *Synapsis.*

It appears that thus far there was no necessity for the paternal and maternal chromosomes in the cells of the hybrid to coöperate to any noticeable extent in functioning, and while some conflicting tendencies may be in operation, each group mixed with the other, seems to have gone on performing its functions normally. Material from both parents undoubtedly prevails in the somatic cells of the mule as it does in the sex cells. Yet these materials in the somatic cells, coming from two vastly different animals, do not in the least interfere with each other physiologically. And while no somatic tissue of the mule has been studied, any suspicion of abnormalities, such as disintegration and decay for example in the muscle cells, could hardly be substantiated by our knowledge of the health, strength, endurance, and longevity of these hybrids. This suggests that developmental processes are also normal. It is highly probable, then, that since the physiological and developmental processes are normal that the mechanism of cell division during the process of development and growth is also normal; the same as it is in the spermatogonial cells where perfect mitotic figures appear and all of the chromosomes divide.

The real conflict ensues during the various stages of the primary spermatocyte as is so plainly evidenced by the numerous abnormalities which occur in these cells. In fact the conflicting tendencies are so great that the destruction of each cell is inevitable, and no spermatozoa are produced, causing the hybrid to be sterile. Up to this stage the paternal and maternal plasmas evidently retain their individuality and would undoubtedly continue to do so were it not for the wonderful phenomena of reduction, which necessitates a fusion of the chromatin components of the germ cells at this stage of maturation, as is known in many of the normal forms in which spermatogenesis was carefully studied.

It is now a well-known fact that in maturation of the germ cells a reduction of the ordinary number of chromosomes to one-half takes place. Before this actual reduction, there is usually a so-called pseudo-reduction, in which the chromosomes unite in pairs so that when the cell is ready for division, although only half of the regular number of chromosomes appear, each is really double or bivalent and equivalent to two of the single or univalent type.

In this hybrid, as was suggested before, it may be supposed that in the somatic cells and in the spermatogonia, the chromosomes from the paternal and maternal animals lie side by side and carry on the customary functions of the cells; but when it comes to an actual fusion of the chromosomes to form the bivalent type necessary for reduction, the incompatibility of the two plasmas renders the pairing difficult and incomplete, or prevents it entirely.

In the horse pairing or pseudo-reduction takes place during the period of synizesis. This period is characterized by massing of the chromatin threads in the center of the nucleus, and later the nuclear wall expands and the entire mass passes to one side of the nucleus leaving a large clear area in the remaining portion (Wodsedalek, '14). This condition is much the same as in the pig (Wodsedalek, '13), except that in that animal the nucleoli were invariably found within the mass of threads and in a position nearest to the nuclear wall; while in the horse the nucleoli are almost invariable within, or next to the clear area. Shortly after the collapse of the chromatin material, the threads pair and appear in apparently half the original number and twice as thick. Later they expand and again occupy the entire contents of the nucleus. Then follows the period of growth, and eventually the threads break up into the bivalent chromosomes which appear in half the original number found in the spermatogonia, plus the accessory.

The pairing of the various components, in the horse, takes place simultaneously, and when the spireme appears it possesses a fairly uniform diameter throughout. This fact indicates clearly that each portion of the spireme is of a bivalent nature, for should some parts remain unpaired or univalent in nature, such parts could be readily discerned by the sudden and noticeable decrease in their diameters. No pairing of parts in the horse was ever observed after the synizesis period, and no chromosomes ever seem to remain unpaired in the primary spermatocyte, with the exception of the accessory which is always unpaired and passes undivided to one pole or the other.

In the mule the period of synapsis is extremely fascinating, and the facts presented here will no doubt stimulate many investigators in the study of hybrid sex cells. The author considers himself exceedingly fortunate in being able to obtain hybrid material which was in such excellent condition for this investigation, especially since the hybrid in question is the offspring of two vastly different parents. Thus far, it appears that there is no case on record among the vertebrates, showing that this important step in the process of maturation of the sex cells of hybrids has been fully and conclusively demonstrated; and since this important phenomenon has never been clearly demonstrated in a hybrid of this nature, and since this material given to me by Dr. Kalkus appeared promising from the start, the author spared no time and energy in making a careful and prolonged study of this particular phase in the maturation of the sex cells of the mule.

It might be stated at the outset that little fusion of the chromatin material takes place, and that there is no definite time for such fusion. As soon as the various chromatin threads in the network of the nucleus become well defined, there appear indications of pairing of some of the threads. This process continues even after the network breaks up and the chromosomes make their appearance (Figs. 8, 9, and 12). Fig. 8 represents an early stage in which two or three of the threads had fused, and a beautiful case of two long threads lying side by side almost across the entire diameter of the large nucleus. Fig. 9 shows a cell in which many more threads had fused and some are still in the process of fusing. Fig. 10 shows a case where only a small amount of fusion had taken place before the parts of the chromatin network had become loose; but the process of pairing seems to have continued in two or three instances regardless of the fact that some of the chromosomes were in the stage of disintegration. Fig. 11 represents a more advanced case, where apparently a great deal of the chromatin material had fused before the network broke up and still there are indications of pairing. Cells of this nature in which a great deal of fusion had apparently taken place, invariably show more or less pronounced indications of decay, and the question arises as to whether this unusual amount of fusion is due to the condition of decay or whether the degeneration sets in because of the unusual amount of fusion. It appears, however, that the great amount of fusion is caused by the existing degenerate condition of the cell in general, for invariably masses of chromatin material bearing no resemblance to normal chromosomes or threads are present in these cells. Fig. 12 shows a still further advanced stage, in which conjugation continues.

There is no regularity in the amount of fusion that takes place in the various cells. In some cells many more chromosomes conjugate than in others. This fact was apparent during the various stages of the comparatively long synaptic period, and became more obvious when numerous accurate counts of chromosomes were made in cells in which all indications of pairing had ceased. The fact that the nucleus expands enormously during the "spireme stage" was a great aid in making many accurate counts. The chromosomes, as a rule, were well segregated with only partial overlapping. Here and there were tubules with cells in exceptionally good condition for this particular investigation, thus rendering possible many definite counts. The number of chromosomes in the different cells at this stage vary considerably. The smallest number found was thirty-four and the largest was forty-nine. This seems to indicate without a doubt that in the few cases where only thirty-four chromosomes could be counted that thirty-two of the fifty-one univalent autosomes had fused. In the few cases where as many as forty-nine counts were made it appears equally as certain that only four of the fifty-one chromosomes had fused. It must be remembered, however, that these two extreme counts were obtained in only a very few cases and stand out as exceptions. All other counts between thirty-four and forty-nine were obtained, but by far the greater majority of counts lie between forty and forty-five, with a fairly good number of thirty-eight and forty-six.

A very interesting as well as important feature about the chromosomes at this stage is the fact that the bivalent ones can as a rule be readily distinguished from the univalents, and the loss of univalents can be accounted for by a proportional increase of the bivalents. If, for example, forty-one univalent chromosomes can be detected, it means a loss of ten others. This loss of ten univalent chromosomes can usually be accounted for by the presence of five large or bivalent chromosomes, making a total of forty-six. If only thirty-one chromosomes can be counted, which means the disappearance of twenty univalents as such, one can usually find ten large bivalent chromosomes, making a total of forty-one. This part of the problem was so fascinating that the chromosomes of hundreds of cells, in which they were well scattered, were carefully counted and an attempt was made to account for the loss or gain, depending on the type of chromosomes that were considered first. In some cases those which appeared to be univalent were counted first and then the bivalents. In other cases those which appeared to be bivalent were counted first and then the univalents, and it is surprising in what a large percentage of cases the necessary number of fifty-one, in terms of univalents, was actually obtained.

Occasionally a discrepancy of one or two, and in rare cases, three bivalent chromosomes, was noted, or in other words, it was sometimes impossible at first sight to distinguish all of the bivalent chromosomes. Even in cases when the individual chromosomes were carefully examined in regard to their single or double nature, one could not always be positive whether he was dealing with a single or a compound body. This fact, however, should not be so very surprising because two of the smallest type of single chromosomes may fuse to form a bivalent one of practically the same size as some of the larger univalent chromosomes. The matter suggests rather strongly that it is not always the same chromosomes that fuse, even in cases where the same counts are obtained. On the other hand there is a possibility of some of the large chromosomes being tri- instead of bivalent, since the fusion of three threads, as well as three chromosomes, was observed in several cases (Fig. 12). In cells in which decay is noticeably under way it frequently occurs that many chromosomes fuse together and form a large body, but this cannot be regarded as fusion in the same sense as synapsis.

It can now be clearly seen that great variation exists in the amount of fusion which takes place in the chromatin material of the various cells in this stage of the maturation process. In some cells fully eight times as many chromosomes pair as in others. The question at once arises why should such a decided variability exist in this respect. It is not very probable that the various cells resulting from the last spermatogonial divisions differ so greatly in their make-up, as no such variability was observed in the spermatogonial cells themselves. The fate of the different primary spermatocytes with respect to the extent of pseudo-reduction of their chromatin material is evidently determined at the time of the formation of the network of the linin threads, for these linin threads seem to persist as the axes of the later chromatin threads which eventually break up into chromosomes.

The nature of the cause of the linin strands connecting with each other in such confusion as they are found, must necessarily remain a matter of conjecture at present. The same force which in normal sex cells at this stage causes the linin strands to come in contact at their free ends, thereby forming long threads, or loops, or a continuous strand, is apparently brought into action in the hybrid cells but not with the same precision. The fact that the two plasmas are so different apparently causes the strands to connect with each other at any point and at any angle, instead of at their free ends only, and thereby forming a continuous network rather than long strands. The tendency of the strands to connect with others, however, must be well pronounced for very seldom can any free ends be seen in the earlier stages (Figs. 8 and 9). Later on, of course, free ends make their appearance but these are caused by the breaking up of the network in parts rather than original failure at fusion. In some cells the end to end fusion of the original components is more pronounced, since many long threads appear in such cases. However, not a single case was seen in which several of the cross-connecting pieces were not present, which condition was never observed to occur in the corresponding stages in the sex cells of the horse.

The fact that the ends of the threads are fused on to some other part of the chromatin network seems to be a drawback in their fusion. Frequently two threads, which have some affinity for each other, and lie in a position parallel to each other and are not too taut, fuse along the central portion with the ends remaining in the shape of V's, because the two threads are attached at each end some distance apart. At times when parts having an attraction for each other are so arranged as to form an acute angle, fusion begins at the point of the angle and continues a short distance, leaving a V at one end. The ends remain unfused at that point, until the network breaks up into chromosomes, when the temporary connections of the two chromosomes in question, too, are severed, and thereby complete their fusion.

There is also considerable evidence that actual migration for purpose of fusion of some parts of the net-work takes place before the connections are entirely severed, and even before there are any noticeable indications of such connections separating, for frequently we find a bivalent thread with both ends still attached to other threads.

3. *Abnormalities in Mitosis.*

The abnormalities in mitosis bear a striking resemblance to the abnormalities found in the sex cells of hybrid pigeons by Guyer ('00). In speaking of abnormalities in pigeons, Dr. Guyer says: "The abnormalities in mitosis are in the nature of multipolar spindles and asymmetrical division and distribution of the chromosomes (Figs. 28–39). These may exist independently one of another, or both may occur together in the same cell. They are more pronounced in sterile birds but may at times be seen in the fertile forms. In very many of the division of primary spermatocytes one or the other, or both of these phenomena are seen. It is a curious fact that the multipolar spindles seem to be confined largely to the primary spermatocytes, and one is prompted immediately to associate the fact with the pseudo-reduction or formation of bivalent chromosomes which occurs normally at this stage of spermatogenesis. The irregularities in chromatin distribution are also seen for the most part in the primary spermatocytes."

The abnormalities in the mule are also in the nature of multipolar spindles and there are attempts at asymmetrical division and distribution of the chromosomes; but in addition to these types of abnormalities we have cases of scattered chromosomes which do not enter into the spindle at all (Figs. 16–36). Guyer's suggestion regarding the curious fact that the multipolar spindles seem to be confined largely to the primary spermatocytes prompts one immediately to associate the fact with the pseudo-reduction or formation of bivalent chromosomes which occurs normally at this stage of spermatogenesis, is considerably strengthened by the results of this investigation on the mule, since a thorough study of the entire phenomena of pseudo-reduction was possible in this hybrid.

The diverse types of multipolar spindles and other abnormalities that occur in the primary spermatocytes of the mule are shown in Figs. 16–36. Fig. 16 represents a polar view of a metaphase stage. Twenty-eight chromosomes, some bivalent and some univalent, can be seen in the equatorial plate, while sixteen all apparently univalent, together with the accessory are scattered about in the cytoplasm. Fig. 17 shows a meager attempt at spindle formation. A bunch of chromosomes is found at the equatorial plate and only a few spindle threads could be seen and those appeared to be broken up. There was no sign of the centrosomes being present. The other chromosomes, both bivalent and univalent, are scattered about. The cell was no doubt in the process of decay. A trivalent chromosome can be seen at the lower part of the figure. Trivalent chromosomes are not uncommon but the three components can seldom be seen at this advanced stage. They usually fuse together, forming a large chromosome, frequently spherical in shape. Especially is this true when the cell is in the process of deterioration. The large spherical body at the upper part of Fig. 18 was probably formed in this way, though it is possible that it is composed of more chromosomes.

Fig. 18 represents a cell similar to that shown in Fig. 17. These cells were found together with many other similar cells in the same tubule, and several tubules bearing such type of cells were observed. All of these cells were characterized by a large clump of chromosomes, which was presumably an attempt at plate formation, and a large number of chromosomes, uni-, bi-, tri-, and even quadrivalent, are scattered about the entire contents of the cell. The cytoplasm of these cells was invariably degenerated; the remains being closely assembled about the large clump of chromosomes, leaving the periphery of the cell clear in appearance. The cell wall, however, retained its normal outline up to this stage of degeneration, which seems to suggest that the clear space was occupied by a fluid. While there were indications of spindle threads more or less pronounced in some cells, many of the cells showed no signs of the presence of such threads (Fig. 18). In such cells they either disappeared or had never been formed. It is a curious fact that such type of cells usually appeared in large numbers in the same locality and that only several tubules bearing them were found, though occasionally single individuals of that type were found in other tubules. The heart-shaped accessory could usually be seen somewhere off by itself (Fig. 18).

Figs. 19–21 show a common type of freak in which the spindle is bipolar and a number of chromosomes did not find place in the equatorial plate but remain scattered about, either somewhere on the spindle or in the cytoplasm. Figs. 19 and 20 each show the large accessory at one pole. In Fig. 21 the accessory could not be easily detected.

Fig. 22 shows an uncommon type of unequal division in which five large chromosomes have passed over to one pole, while about thirty-six remained in the original place. This indicates that the total number of chromosomes in that cell is forty-one and that ten of them, in view of the previous evidence and discussion, must necessarily be bivalent. Each of the five at one pole on account of their size appear to be bivalent. One of them, in which the two parts have not fused along their entire lengths, obviously shows its bivalent nature. Further evidence that these five are double can be based on the fact that only about five of the chromosomes in the big group appear to be double. This shows then that no chromosomes have divided, but that five bivalent ones were pulled over to one pole in their entirety. Each of these groups without further division would in all probability, if decay did not set in too early, form a separate nucleus. And it can be safely assumed that the binucleated condition of cells sometimes seen in this tissue, in which one nucleus is much larger than the other, was brought about in this fashion.

Fig. 23 shows still another type of cell with a bipolar spindle or an attempt at one. A ring of ten chromosomes can be seen near one pole and partly pushed out of the cell through the cell wall which had been broken at that place. Judging by the convergence of the spindle threads in that half of the cell one would conclude that these ten chromosomes never occupied a position in the plate, and it appears as though this was an attempt on the part of the cell to get rid of some of the chromatin material which did not harmonize with the other parts of it. The clump of chromosomes was apparently repelled by the others with sufficient force to cause them to break through the cell wall. Only about twenty other cases similar to the one just described were observed in all of the tissue studied. However, a number of these the author is inclined to regard, on account of circumstantial evidence, as being due to methods of technique, though even in the more suspicious cases he is not absolutely positive that such in the real cause. In at least a dozen of cases there seems to be no doubt but that the expulsion of masses of chromosomes is actually due to the activity of the cell itself. Among these twelve cases a regular series from partial to total expulsion of a mass of chromosomes can be demonstrated. The number of chromosomes comprising such a discarded mass varies from five to nineteen.

The assumption that an effort is made on the part of some cells to discard some of the chromatin material is further substantiated by the fact that in many cells a mass of chromosomes was seen closely applied to the inner side of the cell wall. At times the cell wall bulges out at such a point and has the appearance of being quite taut in the immediate vicinity of the chromosomes, giving the impression that some repelling force is acting upon them. The most notable evidence in favor of this assumption lies in the morphology of the cell, represented in Fig. 24. The unfortunate thing about this matter is, of course, that only a single cell of its kind, the one here represented as accurately as possible, was found even after a long and diligent search for more was made. It may at first seem very unscientific or even ridiculous to attach so much value to a single cell. Nevertheless, the significance of the structure of this cell, lonely as it is, must not be underestimated. The cell is undoubtedly a primary spermatocyte in the telophase stage. The cytoplasm has failed to divide. This is in accordance with all other observations made on primary spermatocytes in this hybrid, since not a single case was noted in which the cytoplasm showed even the slightest indications of division or construction.

Just exactly what the nature of the cell was in its metaphase stage with regard to the arrangement of the chromosomes can not be definitely stated. One thing seems fairly certain, and that is that no division of individual chromosomes took place. For the number of chromosomes can still be fairly accurately determined since they had become only partially disintegrated. A count of the chromosomes in the various parts of the cell reveals a total of about forty-three, fourteen in the oblong nucleus, ten in the spherical one, three in the cytoplasm, and sixteen in the small spherical nucleus outside of the cell proper. The total of forty-three shows that eight chromosomes are lacking to make the total of fifty-one contributed by the last spermatogonial division. In accordance with the prevailing scheme of synapsis in other primary spermatocytes this means that eight of the forty-three chromosomes should be bivalent and thirty-five univalent. In examining the various chromosomes with regard to size, nine of them seem to be noticeably larger than the rest; five are in the oblong nucleus and four in the large spherical one. One of the nine is possibly the accessory, thus leaving the required number of eight bivalent chromosomes. The three left behind in the cytoplasm and the sixteen outcasts are all univalents.

As to the origin of the two nuclei within the cell, several possibilities suggest themselves. The possibility which appears most tenable is that the cell in its metaphase stage resembled the one represented in Fig. 32. It can be seen from that figure that the cell possessed a large spindle, at one side of which was a plate of chromosomes with threads extending only to one pole. In another part of the cell is a group of about twenty chromosomes with no signs of spindle formation. It can be assumed that the two nuclei shown in Fig. 24 were formed from two masses of chromosomes resembling the two plates of chromosomes shown in Fig. 32, and that the nuclei were reorganized without division of the chromosomes. The latter phase of the assumption is based on the fact that indications of the actual division of the chromosomes in the primary spermatocytes are extremely rare, and that occasionally it can be seen that the nuclei actually enter the process of reorganization before the chromosomes had divided. This is especially true in cells with multipolar and double spindles. As to the nucleus outside of the cell (Fig. 24), it may be suggested that the chromosomes contained therein resembled in the metaphase stage of this cell the group of chromosomes without spindle threads represented in Fig. 32, and that later they were expelled from the cell after the fashion shown in Fig. 23. The three chromosomes in the cytoplasm were probably treated with indifference by the three large groups of chromosomes, being neither attracted nor repelled by them, and thus remained scattered about in the cytoplasm. The material necessary to construct a separate nuclear wall for the three deserted chromosomes was apparently lacking.

Another possibility as to the origin of the nuclei in question is that the cell may have had two spindles, as are shown in Fig. 29, with an additional group of chromosomes without the spindle threads, for such cells were also observed. Still another possibility is that the cell may have possessed three spindles like those shown in Fig. 34 and that the chromosomes belonging to one of the spindles were eventually expelled from the cell and there reorganized a nucleus while the other two spindles remained within the cell.

There seems to be no doubt but that the chromatin material found in the small nucleus on the outside of the cell shown in Fig. 24, originally formed a part of the inner contents of the same, for a distinct connection in the form of coarse granular threads still persists between it and the chromosomes within the cytoplasm, as well as the two large nuclei themselves. The group of sixteen chromosomes was no doubt expelled from the cell and there formed a separate nucleus. The nuclear membrane is thin but well defined. It can also be seen that a quantity of cytoplasm was forced out with the chromosomes. Some of it, very finely granular, persists around the nuclear membrane. The rupture in the cell wall occasioned by the expulsion of such a mass of chromosomes had evidently regenerated in this cell. That the cell had begun to degenerate is evidenced by the presence of large vacuoles and other irregularities in the cytoplasm. The signs of decay always seem to be manifest in the cytoplasm first and then in the chromatin.

The fact that more cells of exactly this type could not be identified is no doubt due to decay which almost invariably sets in before the aim of the cell is accomplished, as will be pointed out later. It is evident that at least some of the other cells seemed to act in the same direction but succumbed before the reorganization of the nuclei could take place. Then, too, it seems reasonable to believe that the isolation of a particular group of chromosomes, as a necessary preliminary step to the function of expulsion, depends largely on the relative arrangement of the linin strands representing such a group of chromosomes in the early stages of these cells. According to our knowledge of the extreme variability in the structure of the linin strand network, such a favorable arrangement of the unwelcome chromatin material can possibly be regarded as being accidental.

Judging by the appearance of the chromosomes, the author is inclined to think that the material expelled by the cells is largely that which was contributed by the mother of the hybrid; and while the number of chromosomes so expelled varies in the different cases, nineteen chromosomes including the accessory which is the number presumably of maternal origin were found in a few instances. There were never more than nineteen chromosomes in the expelled group. This, however, is only a matter of suggestion, for the situation can not possibly be cleared up until a careful study has been made of the sex cells of the ass.

Fig. 25 shows a type of cell in which there is one large spindle and a bunch of chromosomes forming a small plate located at right angles to the large one. It can be seen that a number of threads radiate from the chromosomes in the small plate and that some of them extend to the poles of the large spindle. The large chromosome in the upper part of the spindle is the accessory. Fig. 26 shows a peculiar quadripolar spindle. Most of the chromosomes are in the plate of the large spindle and many of them show signs of division. A small group of chromosomes is located at right angles to the right of the large plate, and spindle threads, extending from them and converging at a conspicuous centrosome, could be plainly seen. On the other side some of these chromosomes seem to be connected by threads to the one pole of the large spindle and some to the other. At the upper pole of the large spindle is a group of eleven chromosomes with spindle threads converging at a centrosome near the cell wall. There was a slight indication of threads on the other side passing to the upper centrosome of the large spindle, but these could not be definitely made out since the spindle in question was at a somewhat oblique angle to the large spindle, and the threads on the opposite side were obstructed from view by the group of eleven chromosomes. The centrosomes in this cell are unusually large and conspicuous. The large chrosome at the lower portion of the main spindle is the accessory.

Fig. 27 is apparently another attempt at a double spindle, the small one at right angles to the large. There was no sign of centrosomes. Fig. 28 is a polar view of a cell with two spindles. In each case the small chromosomes are in the center of the plate. Seventeen chromosomes form the plate in one spindle and twenty-five in the other, making a total of forty-two. There then should appear ten large chromosomes, including the accessory in this cell. In the smaller spindle containing seventeen chromosomes, four are undoubtedly bivalent, and in the large spindle of twenty-five chromosomes, six should be larger than the rest, which upon very careful examination, seems to be the case, but some of the other chromosomes are also somewhat larger than usual. This condition is possibly due to the fact that decay is well under way in the cell and the chromosome may have become distorted.

Fig. 29 shows an excellent case of two spindles, one is larger than the other and also contains the larger chromosomes. It is probable that the large spindle is paternal in nature, and the small one maternal.

Fig. 30 shows an unusual freak with the upper part giving the appearance of two closely applied spindles. At the opposite end, however, the threads do not converge at two separate poles nor at a single pole but remain loose. At first sight one gains the impression that the spindle was a tripolar one, with two poles on one side of the double plate and a single pole on the opposite side, and that the single pole was cut off in the process of sectioning. But a careful investigation shows that the cell is entire and that no part of it was cut off. Guyer ('00) represents a similar case in hybrid pigeons, and describes it as a case in which each fiber of the unusually loose spindle seems to terminate at one end in a small centrosome-like dot or granule.

Fig. 31 shows a tripolar spindle which is really a case of two spindles fused together. All of the chromosomes are found on the spindle and some are apparently in the process of division. Fig. 32 shows another type of tripolar spindle and a group of nineteen chromosomes, including the accessory, in the cytoplasm, corresponding to the group of chromosomes which are sometimes expelled from the cell. Fig. 33 shows a degenerate cell with three ragged spindles converging at a common point. The accessory chromosome is off by itself and from it extends a thread toward the point of convergence of the three spindles. The cell is well along in the stage of decadence. Some of the spindle threads are broken up and others seem to have fused together.

Fig. 34 shows a cell with three perfect spindles meeting at a common conspicuous centrosome. The bivalent chromosomes appear to be distributed among the three spindles. Fig. 35 shows an extremely rare type of quadripolar cell with five spindles closely and symmetrically arranged, with three chromosomes remaining loose in the cytoplasm. Fig. 36 shows a cell which is the only one of its kind that was found and is no doubt the anaphase stage of a cell with a quintespindle arrangement similar to that represented in Fig. 35. Eighty-five chromosomes, including the accessory near the centrosome to the left, could be distinguished as represented. Six of the chromosomes in the central spindle evidently had not divided. This in terms of univalence would suggest that there is a total of ninety-one chromosomes in the cell. The accessory remains undivided as it does in the horse in the primary spermatocyte. Without the accessory, then, there would be ninety chromosomes, which suggest rather strongly that forty-five autosomes, of which five were presumably bivalent lined up for division in the metaphase stage of this cell and that all but the six in the center had divided. Only two or three of these six remaining undivided could possibly be taken for bivalents, which fact indicates that the bivalents as well as the univalents had divided. The centrosomes were well defined and there were only slight indications of deterioration in the cell.

The researches of Hansemann ('91), Lustig and Galeotti ('93) and others, show that asymmetrical mitoses are of very general occurrence in carcinoma cells and other pathological tissues; and Schottlander ('88) and Galeotti ('93) among others have shown that similar abnormalities in mitosis may be produced artificially in many tissues by treatment with dilute solutions of various drugs, such as quinine, chloral hydrate, cocaine, potassic iodide and antipyrin.

At first thought, in view of what is known about the effects of drugs on mitosis, it would appear that the abnormalities in division of the primary spermatocytes are probably due to some deleterious chemical substances which may be produced by the degenerative processes going on in the tubules. This however, does not account for the fact that the abnormalities in the mule are practically entirely restricted to the primary spermatocytes. It appears more certain that the abnormalities in this hybrid are due to the conflicting tendencies brought into action within the cells themselves at this stage of spermatogenesis when coöperation between the two parent plasmas becomes necessary but is impossible on account of their vast dissimilarity.

Giant Cells.

Giant cells appear occasionally but do not seem to be as numerous as was described by Guyer ('00) in hybrid pigeons. Fig. 37 represents a large cell of rare occurrence. It appears to be a primary spermatocyte with three spindles in the anaphase stage, but the chromosomes are unusually small and resemble more those of the nurse cells.

Fig. 38 shows an enormous cell with four large nuclei. The cell is evidently a primary spermatocyte in the early prophase, as the size and structure of the nuclei, and the uniform consistency of the cytoplasm are identical to those of normal cells in this stage (Fig. 7). The abnormality appears to have taken place in the last two divisions of a spermatogonial cell; only the nuclei have divided and therefore remained in the same mass of cytoplasm. Primary spermatocytes with four nuclei appear occasionally, but in practically all cases there is evidence that the abnormality had occurred within the cells themselves and not in the spermatogonia.

Fig. 39 is another giant cell in which all of the chromosomes seem to be in the huge non-symmetrical quadripolar spindle. About a hundred chromosomes could be counted in this cell, some of which are apparently the result of division and others are in the process of dividing. There are undoubtedly many more chromosomes present but the exact number could not be determined owing to the position of the large equatorial plate. It is probably a spermatogonial cell which possessed two complete nuclei and the number of chromosomes in excess of one hundred and two may be due to the fact that many of the chromosomes have divided. Parts of the spindle seem to be in the late metaphase and others in the anaphase stage.

Multinucleate Cells.

Primary spermatocytes with nuclei ranging from two to six in number are not uncommon (Figs. 40–43). The most common ones are those with two and four nuclei. Such cells appear to be in the late telophase stage. There is much irregularity in size among the nuclei of the same cell. For example, when two nuclei are present they are sometimes of similar size and then again one is much larger than the other. In cases where three nuclei are present they are sometimes of practically the same size, or form a gradation in sizes, or two large and a small one, or two small and a large one appear, and so on. Frequently one or more chromosomes were left in the cytoplasm while the others formed one or more nuclei. Fig. 41 shows a decaying cell with two nuclei, and a large chromosome left in the cytoplasm but connected with one of the nuclei by a coarse strand. Such strands were not always present. It is possible that the chromosome in question is the accessory, though one of the chromosomes within the nucleus to the left also resembles it.

The formation of more than one nucleus is not at all strange, when we consider the various freakish spindles of the primary spermatocytes and the fact that the cytoplasm of these cells was never observed to be in the process of division. A spindle like those shown in Figs. 34–36 for example, may, if decay does not set in beforehand, form five or six nuclei. A cell like that represented in Fig. 29 may eventually possess four nuclei, one like that shown in Fig. 31 three nuclei, and so on. In all of the comparatively few cases that reach the telophase stage before decaying, we must necessarily expect some such abnormalities.

Destruction of the Germ Cells.

In speaking of degeneration of the germinal cells in hybrid pigeons, Guyer ('10) says: "Degenerative processes were in progress in the testes of all the sterile forms, but were most pronounced in hybrids between very divergent species, or between unlike hybrids from birds which were themselves descendants of fertile hybrids. There were some such extreme cases of degeneration that only the layer of cells lying along the wall remained in the tubule. Where such a degree of degeneration exists there is, of course, no approach to the formation of spermatozoa. There is often a strong invasion of wandering cells into the tubules, especially where the degenerative activity has become extensive. The interspaces between such tubules are also usually packed with cells which have much the same appearance as white blood corpuscles. Little clumps and globules of deeply staining cytoplasm are scattered about among the cells within the tubules. The oval nuclei of the Sertoli cells are also generally to be seen in varying numbers.

"In some places it really looks as if the germinal cells themselves lose their cell walls and characteristic appearance and become leucocytes, but this point will require very careful study before any definite conclusions can be reached. In other tubules the original cells, or cells which have wandered in, settle down and take on exactly the appearance of the large stroma cells which are ordinarily present outside the tubules. The cytoplasm of such cells has a peculiar alveolar-like appearance that is very characteristic."

The destruction of the sex cells in the mule appears to be restricted to the primary spermatocytes, where it seems inevitable. The incompatibility of the two widely different plasmas contributed by the parents renders completion of spermatogenesis in this hybrid impossible and the animal remains sterile. The process of deterioration sets is as soon as coöperation of the chromatin material becomes necessary in the primary spermatocyte. By far the largest amount of destruction takes place in the "spireme stage" before the chromosomes can make their appearance. Sometimes the nucleus in this stage becomes enormously distended and the thin chromatin threads break up into numerous fragments before any noticeable pairing takes place. Then again, the fragmentation takes place after some of the threads fuse (Fig. 10), or after the chromosomes make their appearance (Fig. 44).

The cytoplasm seems to decay first and large masses of naked nuclei in various degrees of decadence can be seen in some of the tubules. Small masses of chromatin material, pieces of threads, cytoplasm, and cell wall, are also abundant in many of the tubules. Occasionally small, naked, though well-defined nuclei are seen. Whether these are formed from cast-off material like the one shown in Fig. 24, or whether they are small nuclei similar to those shown in Fig. 42, retaining their structure after the cytoplasm had fallen to pieces is difficult to determine. The latter suggestion can easily be conceived, and the former is not unreasonable, since there is no logical reason to make one believe that the expelled material invariably remains attached to the cell. A much more legitimate assumption would be that, since the material is cast off by the cell, it ordinarily leaves the cell entirely and becomes free among the other material in the lumen of the tubule. This assumption would tend to explain further the extremely rare occurrence of cells similar to the one shown in Fig. 24.

Figs. 44–52 show various types of degenerating cells. Fig. 44 is in the late prophase, and Fig. 45 in the metaphase showing a partly expelled mass of disintegrating chromosomes. Figs. 46, 48 and 49 show the collapsed condition of the cytoplasm and the dense nature of the nuclei. The nuclei sometimes retain their normal shape but the chromatin material within fuses into a continuous mass, which is often perforated by many vacuoles (Figs. 46 and 49). Occasionally a single large vacuole appears in the center of the nucleus leaving the chromatin material arranged in a layer next to the nuclear membrane (Figs. 48 and 52).

Fig. 47 is a degenerate cell possibly in the late prophase stage in which many of the chromosomes have fused into large spherical bodies which remained scattered about in the cytoplasm. Fig. 50 shows a cell with an ill-formed nucleus and a mass of chromatin material left out in the highly degenerated cytoplasm. Fig. 51 shows a cell in an advanced stage of destruction in all of its parts. Fig. 52 shows a collapsed nucleus with a portion of naked cytoplasm.

On account of the broadcast destruction of the primary spermatocytes in the early stages, a comparatively few are left that attain the metaphase and early anaphase stages (Figs. 18–45) when they, too, meet their fate; and the remaining few that survive the anaphase succumb soon after, and no secondary spermatocytes nor spermatids, and consequently no spermatozoa are formed and the hybrid therefore remains sterile.

The invasion of tubules by wandering cells is not as pronounced in the mule as in pigeon hybrids, as described by Guyer ('00). Even where the degenerative activity was most pronounced the leucocytes were rare or absent altogether. Nor was there any indication of the germinal cells themselves losing their cell walls and characteristic appearance and becoming leucocytes, as sometimes appears to be the case in hybrid pigeons according to the same author.

SUMMARY.

1. The parents of the mule are widely different as can be seen from the comparison of the horse and the ass.

2. The mule partakes of both the sire and the dam, but appears to resemble the ass more than the horse, both in structure and habits.

3. The greatest difference seems to lie in the relative number of chromosomes in the cells of these two animals. The horse has thirty-seven and the mule fifty-one. This suggests that the

number in the ass is about sixty-five, thus making a difference of twenty-eight chromosomes between the parents of the hybrid.

4. The seminiferous tubules of the mule contain a much smaller amount of germ cells than do the tubules of the horse. Some of the tubules of the mule are entirely devoid of sex cells.

5. The sex cells of the mule are larger than those of the horse in the corresponding stages.

6. All of the fifty-one chromosomes in the spermatogonial cells of the mule enter the spindle for division. The mitotic figures are normal and there are no straggling chromosomes.

7. The accessory chromosome of the hybrid, which is undoubtedly maternal in origin, resembles entirely the accessory of the horse, which fact shows that this sex-determining chromosome retains its individuality.

8. The period of synizesis which is so obvious in the primary spermatocytes of the horse, is lacking in the mule.

9. The spireme of the horse is also lacking in the mule, but is replaced by a continuous network of chomatin threads, parts of which sometimes resemble the spireme to a certain extent.

10. There is no definite time for the pairing of threads or chromosomes in the hybrid; the synaptic period begins at the time the chromatin threads make their appearance and continues through the prophase.

11. The pairing of chromosomes, or pseudo-reduction, is always incomplete and very inconstant. The number of chromosomes in the late prophase of the primary spermatocytes varies from thirty-four to forty-nine. The greatest majority of counts of chromosomes lie between forty and forty-five. The expected number, if reduction was complete, would be twenty-five besides the unpaired accessory.

12. In the late prophase of the primary spermatocytes the bivalent chromosomes can as a rule be readily distinguished from the univalent ones. The number of chromosomes which the various cells lack in order to make the original total of fifty-one, in terms of univalence, can usually be accounted for by the proportional increase in the presence of bivalents in such cells.

13. Up to the early prophase of the primary spermatocytes there seems to be no necessity for the paternal and maternal

chromosomes to coöperate in functioning. Each group seems to go on performing its functions normally. The real conflict ensues during the various stages of the primary spermatocyte, and is no doubt occasioned by the necessity for coöperation on the part of the paternal and maternal chromosomes in the process of conjugation or pseudo-reduction.

14. Abnormalities in mitosis occur invariably in primary spermatocytes that attain the metaphase stage.

15. Giant cells are occasionally seen.

16. The chromatoid body which is very conspicuous and constant in the horse is entirely lacking in the mule.

17. There is considerable evidence that primary spermatocytes make an attempt to eliminate some of the chromatin material.

18. The chromosomes expelled by the cells appear to be those which were contributed by the mother of the hybrid.

19. Destruction as well as abnormalities in mitosis seem to be restricted to the primary spermatocytes. Most of the cells disintegrate during the prophase, especially during the period of synapsis. Others meet their fate in the metaphase or early anaphase stages. The remaining few that survive the anaphase succumb soon after, and no secondary spermatocytes nor spermatids, and consequently no spermatozoa are formed and the hybrid remains sterile.

20. There are no authentic cases on record showing that fertility ever occurs in this hybrid.

LITERATURE CITED.

Galeotti, G.

'93 Ueber Experimentelle Erzengung von Unregelmassigkeiten des karyokinetischen Processes. Beitr. z. patholog. Anat. u. z. Allg. Pathol., XIV., 2, Jena, Fischer, 1893.

Guyer, M. F.

'00 Spermatogenesis of Normal and of Hybrid Pigeons. University of Cincinnati Bulletin No. 22.

'10 Accessory Chromosomes in Man. Biol. Bull., Vol. XIX., No. 4.

Hansemann, D.

'91 Karyokinese und Cellularpathologie. Berl. Kln. Wochenschrift, No. 42 1891.

Hayes, M. H.

'04 Points of the Horse. New York.

Lustig and Galeotti, G.

'93 Cytologische Studien ueber pathologische menschliche Gewebe. Beitr. Path. Anat., XIV., 1893.

Lydekker, R.
'12 The Horse and its Relatives. New York.
Montgomery, T. H.
'12 Human Spermatogenesis. Jour. Acad. Nat. Sci. Phlla., XV.
Plumb, C. S.
'06 Types and Breeds of Farm Animals. New York.
Schottlander, J.
'88 Ueber Kern und Zelltheilungsvorgange in dem Endotheil der enzandeten Hornhaut. Arch. f. mikr. Anat., XXX., 1888.
Wilcox, E. V.
'07 Farm Animals. New York.
Wodsedalek, J. E.
'13 Spermatogenesis of the Pig, with Special Reference to the Accessory Chromosomes. Biol. Bull., Vol. XXV., No. 1, June, 1913.
'13 Accessory Chromosomes in the Pig. Science, N. S., Vol. XXXVIII., No. 966, pp. 30–31.
'14 Spermatogenesis of the Horse with Special Reference to the Accessory Chromosome and the Chromatoid Body. Biol. Bull., Vol. XXVII., No. 6, December, 1914.

EXPLANATION OF PLATES.

PLATE I.

(All of the drawings were made with the aid of a camera lucida, × 3,000.)

FIG. 1. Early spermatogonial cell showing a large heart-shaped nucleolus and two small karyosomes.

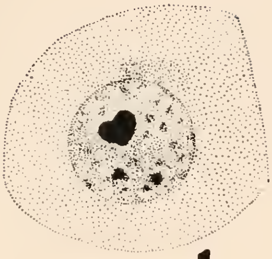
FIG. 2. Resting stage of a full grown spermatogonial cell showing the large triangular or heart-shaped nucleolus and the two spherical karyosomes.

FIG. 3. Late prophase of spermatogonial division showing fifty ordinary chromosomes and the large accessory which can easily be distinguished.

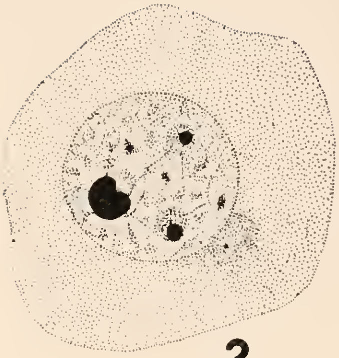
FIG. 4. Metaphase of division in a spermatogonial cell showing a perfect spindle with the accessory dividing at the left.

FIG. 5. Anaphase of division of a spermatogonial cell showing a perfect mitotic figure.

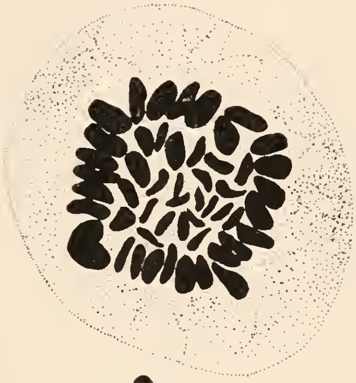
FIG. 6. Late anaphase of division of a spermatogonial cell with perfect mitosis again in evidence.



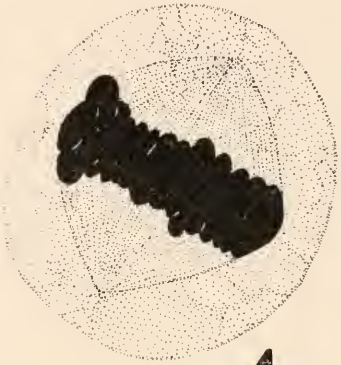
1



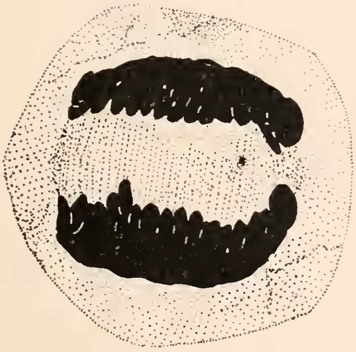
2



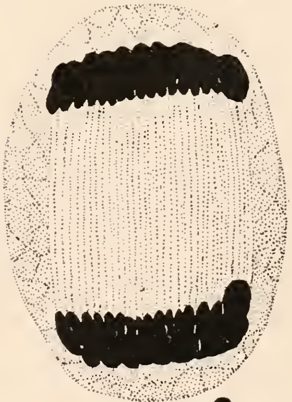
3



4



5



6

J. E. WODSEDALEK.

Plate II.

Fig. 7. Resting stage of a primary spermatocyte showing the characteristic heart-shaped nucleolus and the karyosomes from which radiate fine linin strands.

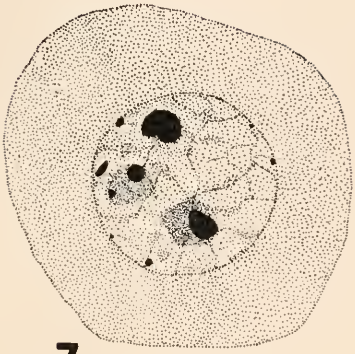
Fig. 8. Primary spermatocyte showing a continuous network of chromatin threads, some of which have paired and others are in the process of fusion, but most of them are unpaired.

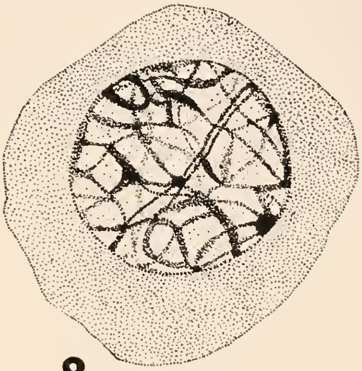
Fig. 9. Primary spermatocyte showing a continuous network of chromatin threads, an unusual number of which have fused; some are still unpaired while others are in the process of fusing.

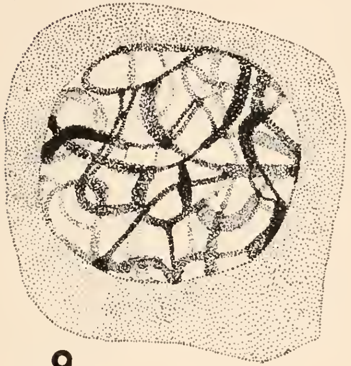
Fig. 10. Primary spermatocyte in process of degeneration in the "spireme stage."

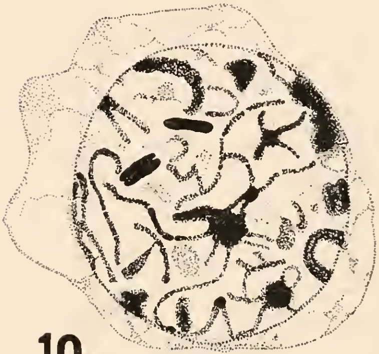
Fig. 11. Primary spermatocyte in the late "spireme stage," showing signs of paired and pairing threads, paired and single chromosomes, and also signs of degeneration.

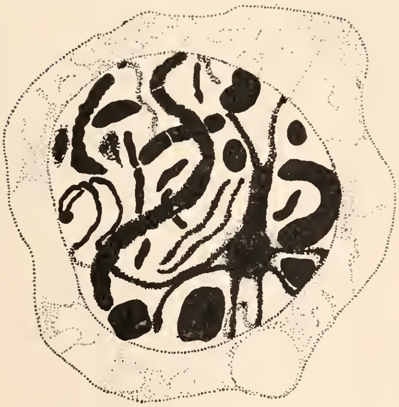
Fig. 12. Late prophase of primary spermatocyte showing univalent and bivalent chromosomes, and one trivalent chromosome. Some of the univalent components are still attached to each other at various angles. The accessory chromosome is very distinct.

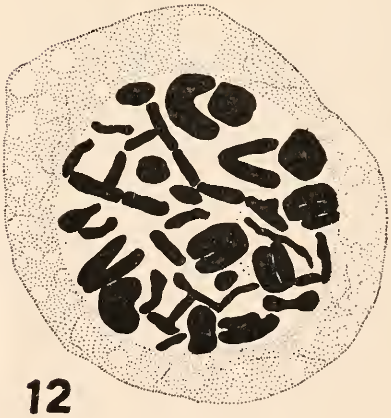

7


8


9


10


11


12

J. E. WODSEDALEK.

PLATE III.

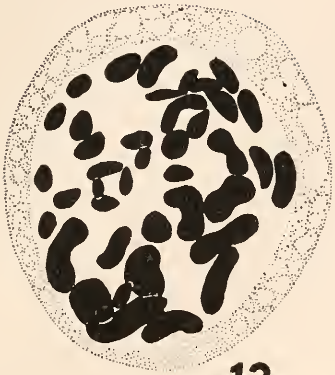
FIGS. 13–15. Late prophase of primary spermatocytes after the partial synapsis had taken place.

FIG. 13 shows forty chromosomes, about eleven of which appear to be bivalent.

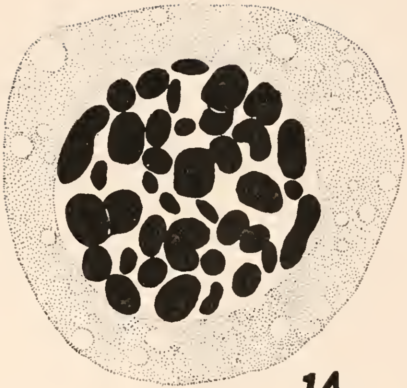
FIG. 14 shows thirty-eight chromosomes about thirteen of which are bivalent. and Fig. 15 shows forty-three chromosomes about eight of which are bivalent Note the heart-shaped accessory in each of these cells.

FIG. 16. Polar view of a metaphase stage of a primary spermatocyte, showing twenty-eight chromosomes in the plate, and seventeen including the accessory are scattered in the cytoplasm.

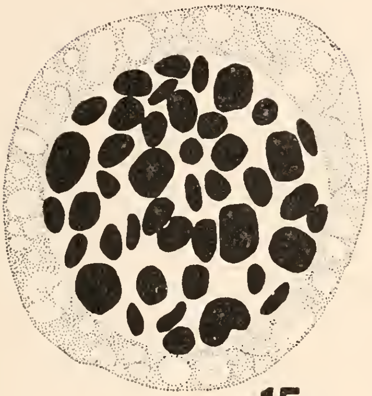
FIGS. 17 AND 18. Degenerating primary spermatocytes in the metaphase stage showing many scattered chromosomes and the remains of the decayed cytoplasm congregated about the plate or large clump of chromosomes.



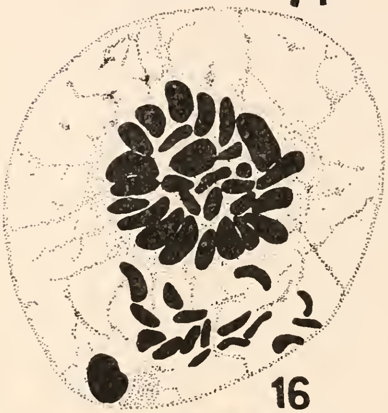
13



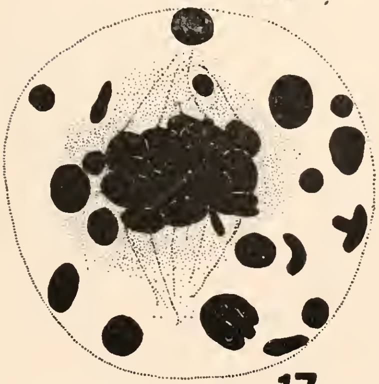
14



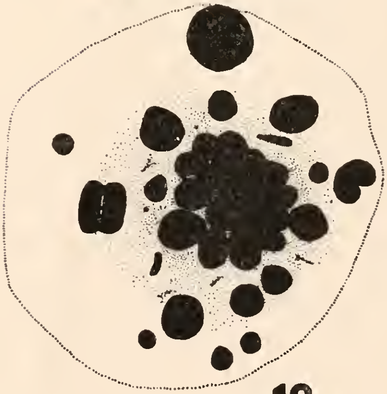
15



16



17



18

J. E. WODSEDALEK.

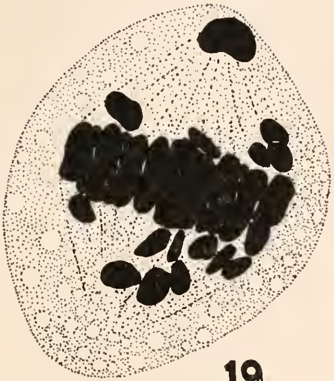
PLATE IV.

FIGS. 19–21. Freak types of primary spermatocytes in the metaphase stage showing most of the chromosomes in the equatorial plate and several scattered about on the spindle or in the cytoplasm.

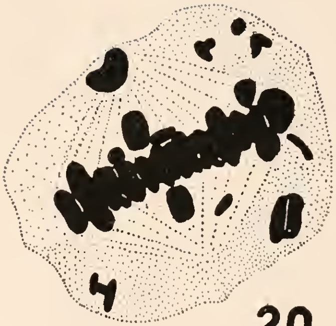
FIG. 22. Primary spermatocyte showing an uncommon type of unequal division in which five large chromosomes have passed over to one place while about thirty-six remained in the original place.

FIG. 23. A somewhat degenerate metaphase stage of primary spermatocyte showing the expulsion of a bunch of chromosomes.

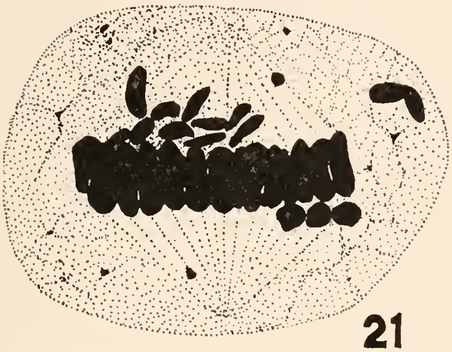
FIG. 24. A late telophase of a trinucleated primary spermatocyte. The nucleus on the outside of the cell wall was apparently formed from a group of chromosomes which were expelled by the cell.



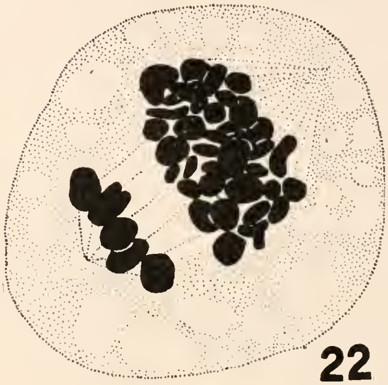
19



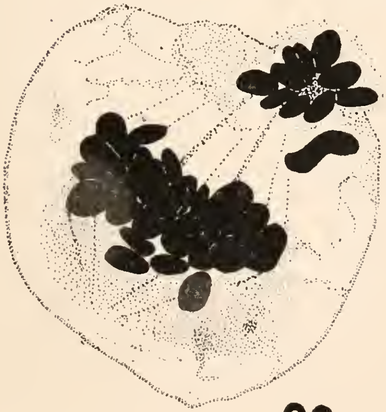
20



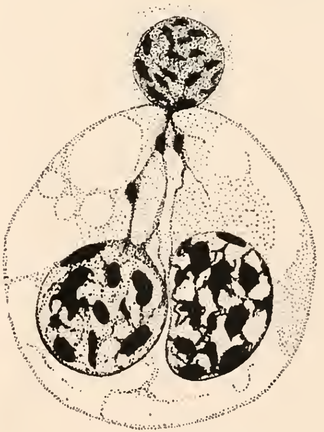
21



22



23

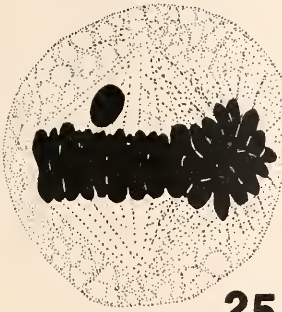


24

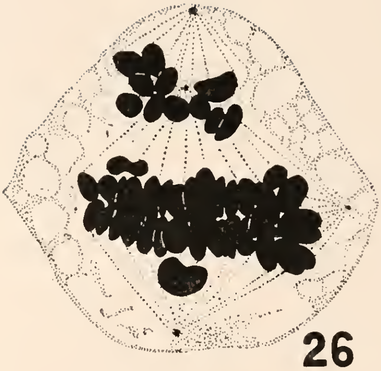
J. E. WODSEDALEK.

PLATE V.

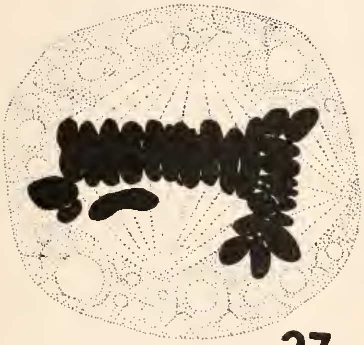
FIGS. 25–30. Abnormal mitotic figures of primary spermatocytes (fully explained in the text).



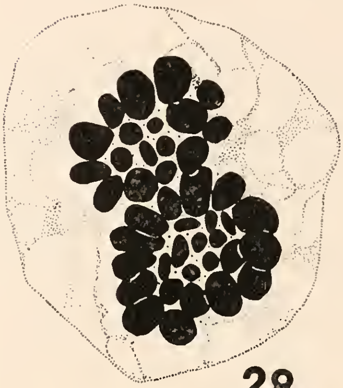
25



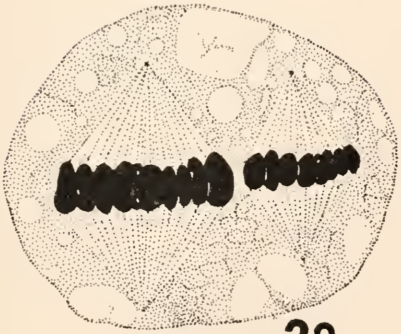
26



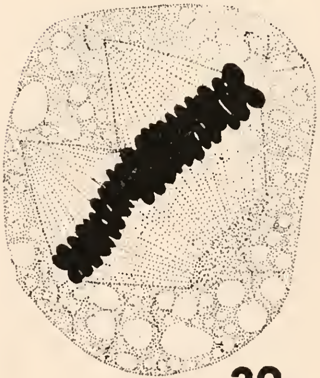
27



28



29

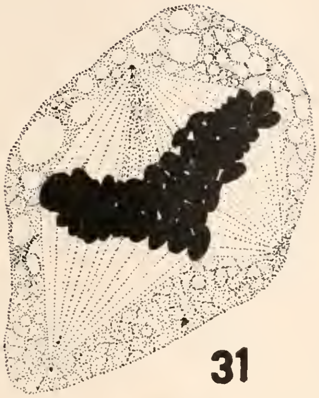


30

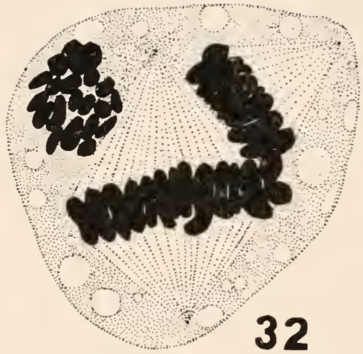
J. E. WODSEDALEK.

PLATE VI.

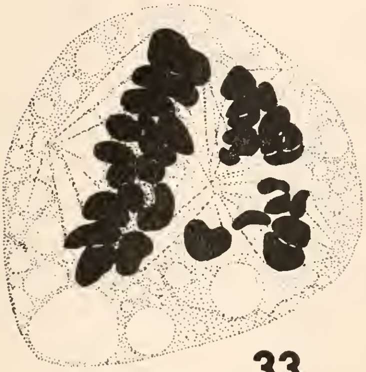
FIGS. 31–36. Abnormal mitotic figures of primary spermatocytes (fully explained in the text).



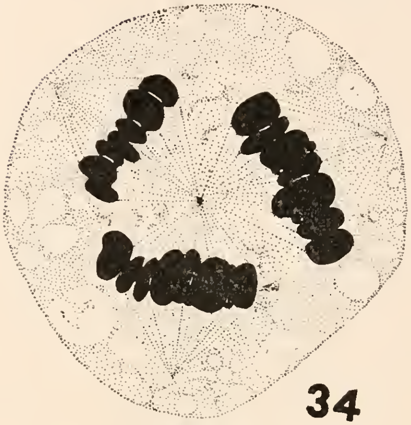
31



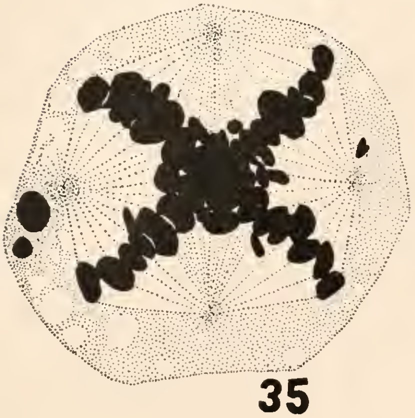
32



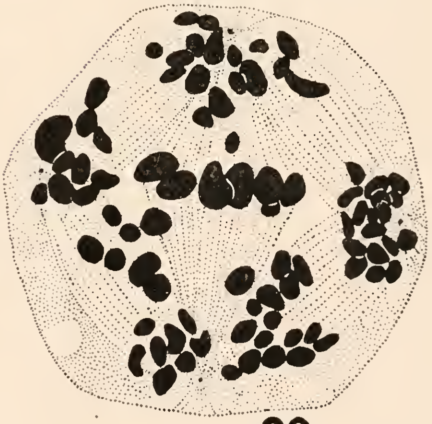
33



34



35



36

J. E. WODSEDALEK.

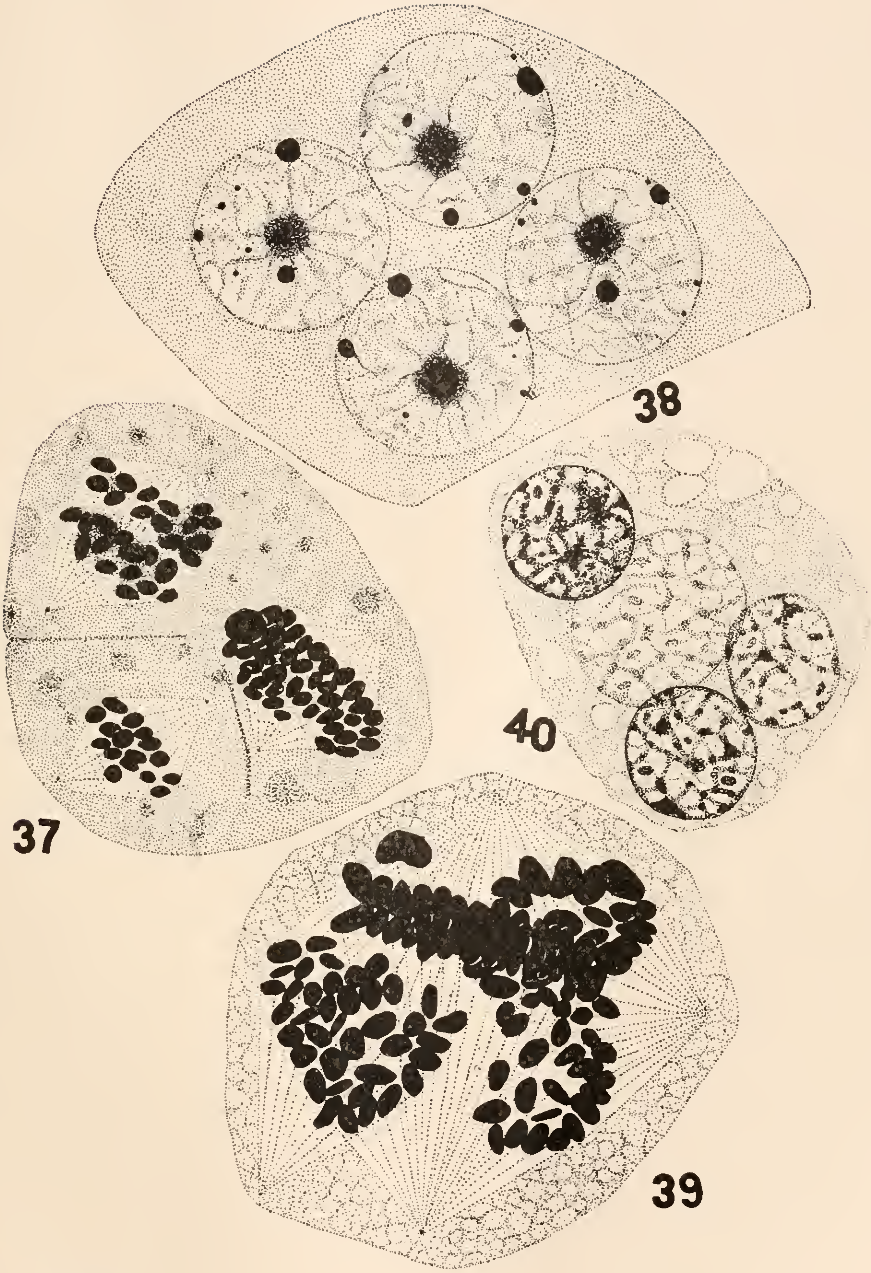
PLATE VII.

FIG. 37. A giant cell with three spindles in the early anaphase stage.

FIG. 38. A giant cell with four large nuclei, each resembling the nucleus of the primary spermatocyte. (See Fig. 7.)

FIG. 39. A giant cell with a huge non-symmetrical quadripolar spindle containing over one hundred chromosomes. It is probably a spermatogonial cell which possessed two complete nuclei and the number of chromosomes in excess of one hundred and two may be due to the fact that many of the chromosomes have divided.

FIG. 40. A quadri-nucleated cell.



38

37

40

39

J. E. WODSEDALEK.

PLATE VIII.

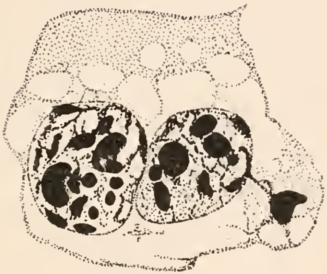
FIG. 41. A bi-nucleated cell in process of degeneration showing a chromosome, possibly the accessory, which was left out in the cytoplasm.

FIG. 42. A cell with six well-defined nuclei.

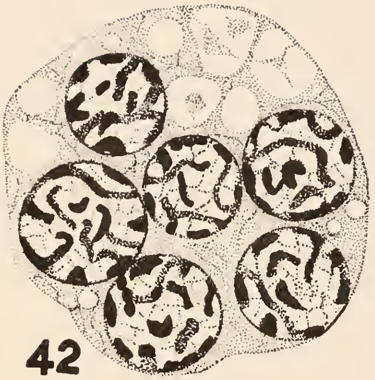
FIG. 43. A degenerating cell with three nuclei.

FIGS. 44–46. Cells in process of degeneration (explained in the text).

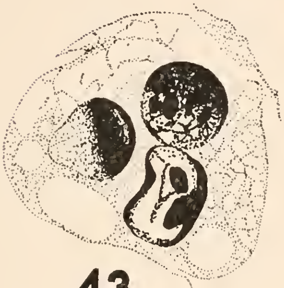
PLATE VIII.



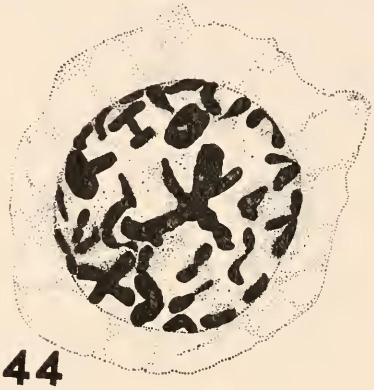
41



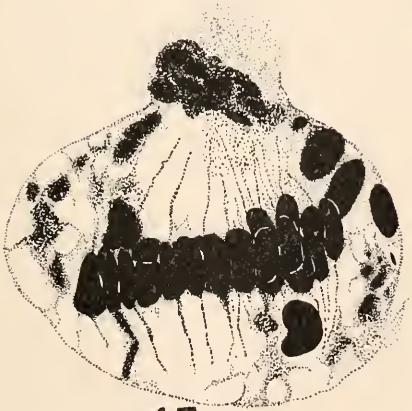
42



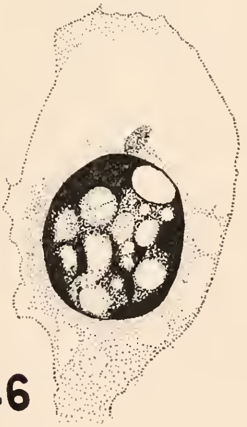
43



44



45

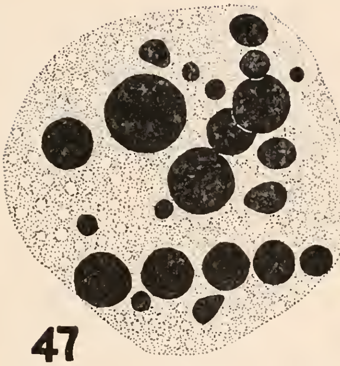


46

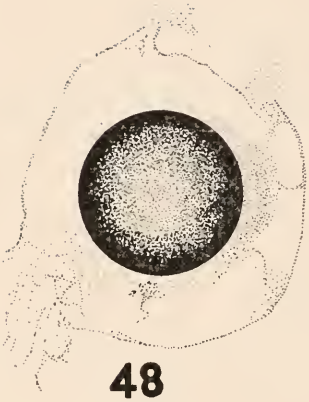
J. E. WODSEDALEK.

PLATE IX.

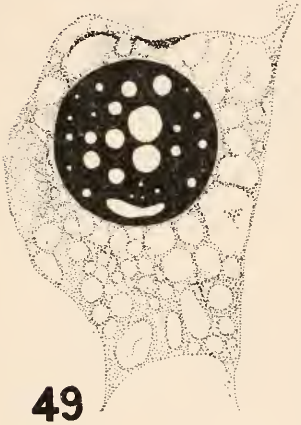
FIGS. 47–52. Various types of cells in process of degeneration (explained in the text).



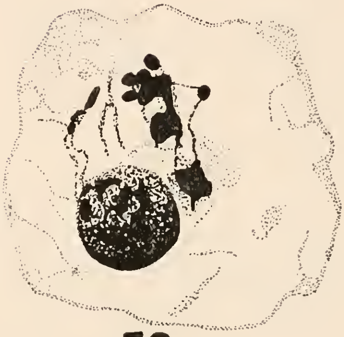
47



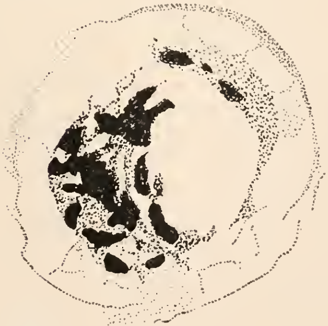
48



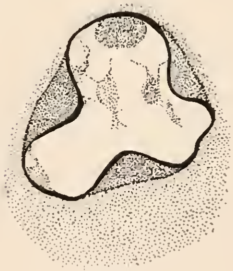
49



50



51



52

J. E. WODSEDALEK.

STUDIES ON THE GEOTROPISM OF THE MARINE SNAIL, LITTORINA LITTOREA.[1]

SAKYO KANDA.

CONTENTS.

I. Introduction 57
II. Material and Methods 58
III. Experiments 61
  1. Preliminary Experiments with Gravity and Light 61
  2. The Relation between the Pressure of Gravity and the Precision of Orientation 63
  3. What Determines whether the Head End will be Directed Up or Down? 66
  4. The Effects of Light and Surface-Film of Seawater 71
  5. The Effect of the Surface-Film of Sea-water 74
  6. The Effect of Chemicals 76
IV. Discussion 76
V. Summary and Conclusions 82
VI. Bibliography 83

I. INTRODUCTION.

In 1888, Loeb pointed out that "Die Schwerkraft der Erde, wenn sie senkrecht gegen die ventrale Seite der Schabe gerichtet ist, wirkt als Reiz, der dieselbe zu Bewegungen veranlasst" (8, p. 9). Since then, with modifications of his method, Davenport and Perkins (2) and Frandsen (3) have investigated the same problem on a slug, *Limax maximus*. The former drew the conclusions that, "the precision of orientation of the slug varies directly with the active component of gravity" (2, p. 105) and that "this tendency (to go either up or down) must be ascribed to some internal condition of the individuals, for it varies in different individuals and in the same individuals at different times" (2, p. 110). The latter reached rather different conclusions, thus: "The different geotactic response, on a glass plate, of different individuals is due mainly to two factors: (*a*) The quantity and quality of the slime secreted, which is a very important factor; (*b*) the relative proportions of the length of the anterior and posterior regions of the animal's body. All the conditions being the same, it is this factor which 'determines whether the head end will be directed up or down'" (3, p. 205).

---

[1] From the Marine Biological Laboratory, Woods Hole, Mass., and the Physiological Laboratory, University of Minnesota, Minneapolis, Minn.

The reactions of the marine forms of *Littorina littorea*, *L. rudis*, etc., to light and other influences have been studied by Mitsukuri (12), Bohn (1), Haseman (4), and Morse (14). Unfortunately, however, none of them has taken into consideration the response of the animals to gravity, although the effect of gravity upon *Littorina* and upon gastropods in general is remarkable and easily observed at the seashore.

Following the example of Frandsen and of Davenport and Perkins, an attempt was made by the writer with *Littorina littorea* to determine: (1) "What relation exists between a variation in the pressure of gravity and the precision of orientation?" and (2) What "determines whether the head end will be directed up or down?"

The experimental work was done in the physiological department of the Matine Biological Laboratory at Woods Hole, Mass., during the summers of 1912 and 1913. The results obtained in 1912 used in this paper are indicated by the date, "1912." The others were all secured in 1913.

II. Material and Methods.

1. *Material.*—The animal used in all the experiments was a marine snail, *Littorina littorea*, which is numerous about Woods Hole. The snails were collected by the writer in the morning or afternoon, just before a new series of experiments was carried on, and were kept in a large glass dish in running sea-water during experiments. The size of the animal used was about 1.5 x 1.1 cm.[1] It was found that this was the more convenient size for experimental purposes, because the bigger, *i. e.*, older, ones retreated into and remained for a long time in their shells when handled. The younger animals are more active and quicker to respond to stimuli.

The same individuals could not be used throughout any series of experiments; since their movements became abnormal, due possibly to fatigue. The same individuals could be used only for four or five trials. Ten individuals, sometimes 11 or 12 (with anticipation of falls), were used for each trial of a series of experiments. As will be seen in the tables, however, very often less than 10 individuals were left on the support for observation, the rest having fallen down.

---

[1] The laboratory collector, Mr. Gray, told me that the snails which I used were about one year old.

2. *Methods.*—Loeb's method (8) modified by Davenport and Perkins (2) and by Frandsen (3), of the different angles of inclination of a support, on which the animals are to be placed, was adopted with variations. The support was a plain glass plate about 22 x 19 cm., marked off on one side into squares of one sq. cm. each, whereby it could be readily determined how far the animals moved from their original places.

The support, or plate, was placed in an apparatus by which it could be held at any ten-degree angle between the horizontal and vertical (see Fig. 1).

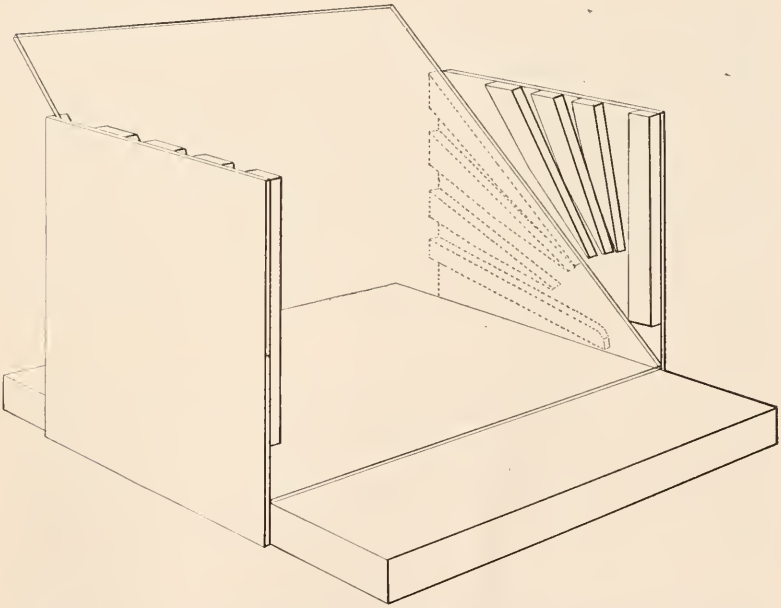


FIG. 1. *G. P.* = a plain glass plate. The rest of the apparatus is all wood.

The animals were placed on the unlined side of the plate, moistened and held upside down at an inclination, say 10° to the horizontal, so that their heads were all directed upward. Meanwhile sea-water was poured, two or three times, over the animals and the plate surface to prevent their moving before the commencement of the experiment, and at the same time to get them to stick tightly to the surface. When the desired number of individuals had been so placed, the support was reversed, and placed at the desired angle in the holding apparatus, so that their heads were, now, directed downward. The animals being negative to gravity, this procedure was necessary in order to determine their movements of orientation in a desired time.

Experiments were conducted either in sea-water in a glass aquarium, or in air. To exclude the effect of light in either case, a square box painted black inside was employed to cover the whole arrangement described above.

After the desired time for a particular experiment, the cover-box was removed. Then the movements which the animals had made were recorded (with detailed notes) as nearly as possible as follows: A movement of 180° from the original was designated as "oriented"; 90° as "horizontal"; and 0° as "original." If an animal was observed to have crawled downward from the original place, it was recorded as positive to gravity. It was noticed that some individuals did not crawl downward quite vertically, but no discrimination as to these is made in the tables. Quite a few individuals, that crawled horizontally, are also arbitrarily classified under "horizontal," although such movements are believed by the writer to have no great significance.

A question might be raised about middle spaces between 180° and 90°, and also between 0° and 90°. Such positions were seldom observed; but if any were observed, they were recorded as "oriented" at a position between 90° and 180°; as "horizontal" between 90° and 45°; and as "original" between 45° and 0°. Moreover, since quite a number of individuals would possibly have become oriented if they had been given longer than one minute, it might have been better to describe them as "orienting" rather than "horizontal." The animals whose position was specified as "original" should not be interpreted as indifferent to gravity. They simply failed to respond to it during the time when they were under experimentation. The time factor was important. This will be confirmed by the following preliminary experiment *A*. But for the more detailed study, the shortest possible time was adopted, to avoid fatiguing the animals.

In all the following tables, the signs indicate positive either to gravity or light by + and negative by −.

III. EXPERIMENTS.

1. *Preliminary Experiments with Gravity and Light.*

Preliminary Experiment A.—A tall beaker full of sea-water was placed upside down in a large dish filled with sea-water. Special care was taken to exclude air bubbles from the beaker. It was then supported on two pieces of glass-tubing about 4 mm. in diameter in the dish in order to let the sea-water within communicate freely with the outside at the bottom. By means of glass-tubing, sea-water was run into the dish so as to have fresh sea-water always at the bottom, but so as not to let any air bubbles into the beaker. Thirty selected snails were placed under the beaker, and between the two pieces of the glass-tubing, which was not high enough to permit the snails within to crawl out from under the beaker. The whole arrangement was then covered with the box already described, to exclude light.

About ten minutes later it was found that all the snails had crawled up the vertical (inside) wall of the beaker and gathered at or near its top. The sea-water would be expected to be fresher and better supplied with oxygen at the bottom than at the top. The snails, however, crawled upward just the same. This was repeated several times, but there was no exception to this rule.

Such results indicate that the upward movements of the snails are not caused by the lack of oxygen but by gravity. This is also fully supported by the fact that the snails crawl upward on moist rocks, or on a moist glass plate, in air where the amount of oxygen does not vary.

Moreover, in leaving the horizontal bottom of the dish to take positions on the vertical wall of the beaker, the snails must have oriented themselves against the pull of gravity. This orientation of the snails cannot be explained by the mechanical theory of geotropism. It is an active process, one of true response to stimulation. Loeb's conclusion which was already referred to, is thus confirmed.

Preliminary Experiment B.—According to Bohn and Mitsukuri, *Littorina littorea* is negatively heliotropic. To test their results, a simple method of "righting" experiments was adopted as follows:

Ten selected individuals were placed dorsal side down on each of two glass plates. The one was covered with the box, and at the same time, the other was exposed to diffused daylight, both for five minutes. The two lots were alternately covered and exposed to daylight, after the animals had been refreshed in sea-water and again turned "on their backs." The results given in Table I. show that light considerably affects the "righting" response of the animals.

TABLE I.

THE "RIGHTING" OF SNAILS ON A HORIZONTAL GLASS PLATE IN DARKNESS AND DAYLIGHT FOR FIVE MINUTES (1912).

No. of Trials.	No. of Animals.	Darkness.		No. of Animals.	Daylight.	
		Righted.	Not Righted.		Righted.	Not Righted.
1	10	4	6	10	3	7
1	10	8	2	10	2	8
1	10	6	4	10	3	7
1	10	5	5	10	2	8
1	10	6	4	10	3	7
1	10	6	4	10	2	8
1	10	4	6	10	4	6
1	10	4	6	10	2	8
1	10	5	5	10	3	7
1	10	4	6	10	2	8
Total. . 10	100	52 or 52%	48 or 48%	100	26 or 26%	74 or 74%

In explanation of this "righting" reaction of the snails, it may not be out of place to refer to Loeb's analysis in the starfish. According to him, the "righting" of the starfish is a stereotropic phenomenon, but not geotropic (10, pp. 64–65). Recently Moore confirms this conclusion through experiments (13, p. 237), while Jennings seems to have been misled in this respect (5, pp. 120–148). The "righting" of the snails may also be a stereotropic phenomenon, though it makes no difference for the present purpose. In fact, however, as will be seen later, contact stimuli interfere with the snails' reaction to gravity. The main point in regard to Preliminary Experiment *B* is that light is a factor in the behavior of these animals.

Preliminary Experiment C.—According to the "mechanical theory" of geotropism, an animal becomes oriented head up because of its heavier posterior region, or it orients itself with its head down, because of its heavier anterior region or head. An experiment was, therefore, made to determine which region of the snails, the anterior or the posterior, was heavier. This was simple. When an individual was placed on a glass plate in sea-water with its dorsal side down, it came out of the shell, and made an "effort" to "right." It was then carefully dropped and watched as it sank, before it retreated into the shell. Every one of them sank with its anterior region up, as expected. The was done also with those individuals which were positive to gravity at a certain angle of inclination. There was, however, no exception.

With the same point in mind, empty shells were tested. One of them was fixed by its ventral side on a small square cover glass, with as little glue as possible. At the three corners of the cover glass, fine threads were fastened. The center of gravity was found by suspension to be located somewhere in the posterior region. This was done with a number of shells and the same results were obtained in every case. These results, therefore, agree with the other observations, indicating that the posterior region of the snails is heavier than the anterior region.

From the above results, the writer is justified in concluding that the center of gravity of the snails is located in the posterior region; and a possible inference from this conclusion is that the posterior region of the snails has a greater specific gravity than the anterior region. This fact and inference may have a bearing in deciding whether the orientation of the animals against gravity is due to purely physical or mechanical pull. This question will be considered throughout all the following experiments.

2. *Experiments to Show the Relation between the Pressure of Gravity and the Precision of Orientation.*

Experiment A.—Davenport and Perkins asked and answered by experiment: "What relation exists between a variation in the pressure of gravity and the precision of orientation of the slug?" (2, p. 100). An attempt was made by the writer to determine experimentally the same question for *Littorina littorea*. The experiments were conducted in sea-water. Light was excluded by means of the cover-box. The results given in Table II. show that the higher the angle the larger is the number showing *negative* geotropism, and the lower the angle the larger the number of (so-called) *positively* geotropic animals.

TABLE II.

GEOTROPISM OF SNAILS AT THE DIFFERENT ANGLES OF INCLINATION OF A GLASS PLATE IN SEA-WATER IN TOTAL DARKNESS.

At beginning of experiments each head pointing downward. Table shows results after one minute.

Temp. of Sea-water.	Angles.	No. of Trials.	No. of Animals.	− Geotropism.				+ Geotropism.		Did Not Move.	
				Oriented.		Horizontal.		Crawled Downward.			
				No.	%.	No.	%.	No.	%.	No.	%.
18½°–21° C.	90°	35	300	300	100	0	0	0	0	0	0
18½°–18½° C.	78¾°	22	200	200	100	0	0	0	0	0	0
18½° C.	67½°	22	200	190	95	3	1.5	5	2.5	2	1
19°–19½° C.	56¼°	23	200	180	90	9	5.5	7	3.5	4	2
19¼°–19½° C.	45°	25	200	163	81.5	13	6.5	12	6	12	6
19½°–20° C.	33¾°	32	200	152	76	15	7.5	16	8	17	8.5
19½°–20° C.	22½°	33	200	130	65	22	11	23	11.5	25	12.5
20°–20¼° C.	11¼°	23	200	110	55	26	13	34	17	30	15
Total.		215	1,700	1,513 or 89%				97 or 5.7%		90 or 5.2%	

As already pointed out, however, it is misleading to consider that the negative geotropism of the snails reaches zero on the horizontal surface of the earth. The force of gravity stimulates the snails, when it happens to pull along the line of the dorso-ventral axis of the animals, that is, on the horizontal surface, as the preliminary experiment *A* has shown. But the larger the angle the shorter the time required for orientation. This is shown in the "horizontal" column, when the number of the animals, most of which were "orienting" at the end of one minute, decreases as the angle of inclination to the vertical increases.

Experiment B.—It has been supposed that hydrostatic pressure affects the reaction of an animal to gravity. To decide this question, a series of experiments was made in air. Light was excluded as usual. The results given in Table III. show an interesting difference compared with those of Table II., which must not be overlooked. The number in the negative geotropism column in Table III. is always higher than the corresponding one in Table II., and that in the positive column in the former is always lower than that in the latter. A possible explanation of these differences is found in the buoyancy effect of the water, which decreases the weight of the animals. In consequence, the pull of gravity is more effective on the animals in air. It would seem therefore that the pull of gravity on the animal *as a whole* must be one factor in its orientation.

Table III.

Geotropism of Snails at the Different Angles of Inclination of a Glass Plate in Air in Total Darkness.

At beginning of experiments each head pointing downward. Table shows results after one minute.

Temp. of Room.	Angles.	No. of Trials.	No. of Animals.	— Geotropism.				+ Geotrop.		Original.	
				Oriented.		Horizontal.		Crawled Downward.		Did Not Move.	
				No.	%	No.	%	No.	%	No.	%
21¾°–22½° C.	90°	20	100	100	100	0	0	0	0	0	0
25° C.	78¾°	14	100	100	100	0	0	0	0	0	0
22½°–23° C.	67½°	15	100	100	100	0	0	0	0	0	0
23° C.	56¼°	13	100	92	92	2	2	2	2	4	4
23½° C.	45°	13	100	86	86	6	6	6	6	2	2
21½° C.	33¾°	17	100	80	80	8	8	9	9	3	3
22¾°–23° C.	22½°	13	100	67	67	13	13	18	18	2	2
22° C.	11¼°	14	100	60	60	13	13	27	27	0	0
Total.		119	800	727 or 90.8%				62 or 7.7%		11 or 1.3%	

The above fact reminds the writer of the "mechanical theory" (6, pp. 2–13) and, also, the "resistance or weight theory" (6, pp. 14–20) of geotropism in *Paramecium*. If the greater weight of snails in air causes the quicker orientation of the animals, and also if the posterior region of the animals is heavier than the anterior region (as has been proven to be the case), the theories above mentioned are favored to some extent. Nevertheless, no mechanical theory can satisfactorily explain why "positive geotropism" increases with decrease in the angle of inclination. This, if a real gravity response, must be explained on the basis of physiological conditions, as Loeb pointed out many years ago.

The question, however, arises: Is there any maximum or minimum of resistance or weight which makes the snails crawl either up or down? This question will be answered later.

Although differences exist between Tables II. and III., the results in the latter table in general show a fair uniformity with Table II. The hydrostatic pressure,[1] therefore, seems seldom, if ever, to affect the negative reaction of the animals to gravity. These two tables also show a fair agreement with the results obtained by Loeb (8, pp. 6–8), Davenport and Perkins (2, pp. 100–105), and Frandsen (3, p. 198), that is, a steady increase in negative geotropism with increase in the angle of inclination. Davenport and Perkins's view in this respect is fully confirmed (2, p. 105).

3. *What Determines Whether the Head End Will be Directed Up or Down?*

Experiment A.—Instead of the plain glass plate used for experiments hitherto, a ground-glass plate was employed for the following experiments, for two reasons: (1) Since the animals live along a rocky seashore, the plain glass plate may not be a proper support for them; and (2) the animals being positively stereotropic, it was thought well to test the effect of contact stimuli upon their geotropism. Light was excluded in these experiments as usual. In the results given in Table IV. it is interesting to note, as expected, a decided decrease in the number of negatively geotropic and an increase of "positively geotropic" individuals with decrease in the angle of inclination. Moreover, below the 45°, no individuals were in their original position.

The animals can stick better to the ground plate than they do to the plain glass. In consequence, they can resist the pull of gravity better on the former than they do on the latter. If so, this serves to indicate that resistance plays a part, particularly in negative geotropism. The resistance theory, however, does not explain why the animals leave the horizontal surface on which they are placed and crawl upward on the vertical wall of a beaker (see preliminary experiment). This fact is unexplained by any mechanical or resistance theory.

---

[1] On preparing Mr. Kanda's Ms. for publication it strikes me that the chief difference between the experiments shown in Tables II. and III. lies in the buoyancy rather than the pressure factor. Remembering that the air pressure must be added to that due to any depth of water, the difference in pressure in Table II. as contrasted with III. is almost negligible. But the weight of the animal in water is much less than in air. The slight difference between Tables II. and III. is to me strong evidence against the buoyancy theory as playing any large part. Mr. Kanda is in Japan and I cannot call his attention to these points, so I venture to add this footnote. —E. P. LYON.

TABLE IV.

GEOTROPISM OF SNAILS AT THE DIFFERENT ANGLES OF INCLINATION OF A GROUND-GLASS PLATE IN AIR IN TOTAL DARKNESS.

At beginning of experiments each head pointing downward. Table shows results after one minute.

Temp. of Room.	Angles.	No. of Trials.	No. of Animals.	– Geotropism.				+ Geotropism.		Original.	
				Oriented.		Horizontal.		Crawled Downward.		Did Not Move.	
				No.	%.	No.	%.	No.	%.	No.	%.
22½°–22¾° C.	90°	14	100	100	100	0	0	0	0	0	0
22° –22½° C.	78¾°	16	100	96	96	0	0	2	2	2	2
22¾°–23° C.	67½°	13	100	82	82	6	6	10	10	2	2
23° –23½° C.	56¼°	13	100	71	71	10	10	16	16	3	3
27° C.	45°	12	100	63	63	12	12	25	25	0	0
25¼° C.	33¾°	11	100	53	53	13	13	34	34	0	0
24¾°–25½° C.	22½°	10	100	39	39	20	20	41	41	0	0
24¾°–25¾° C.	11¼°	10	100	23	23	22	22	55	55	0	0
Total		99	800	610 or 76.2%				183 or 22.8%		7 or .8%	

Experiment B.—A few experiments were made to determine the effect of a dry surface at the angle of 90°. The animals experimented on were carefully dried on filter paper. They were placed on a dry smooth glass plate with their heads directed *upward.* Light was excluded and the animals examined after two minutes. The movements of the animals on the plate were readily seen by the tracks of the mucus secreted by them.

Of 60 animals used 31 dropped off, 6 or 15 per cent. of the remainder oriented and crawled down, 31 or 79 per cent. crawled upward and one crawled horizontally. The striking fact is the number of individuals that oriented *positively* and crawled down. This evidence goes to show that the animals tend to become positive on a dry surface, which would evidently serve as a protective reaction when they are left on rocks by a retreating tide.

The movements of the animals on the dry plate were regular in some cases and irregular in others. The figures given are, therefore, somewhat arbitrary. To avoid this inaccuracy, a few typical examples of movement are shown in figures as follows:

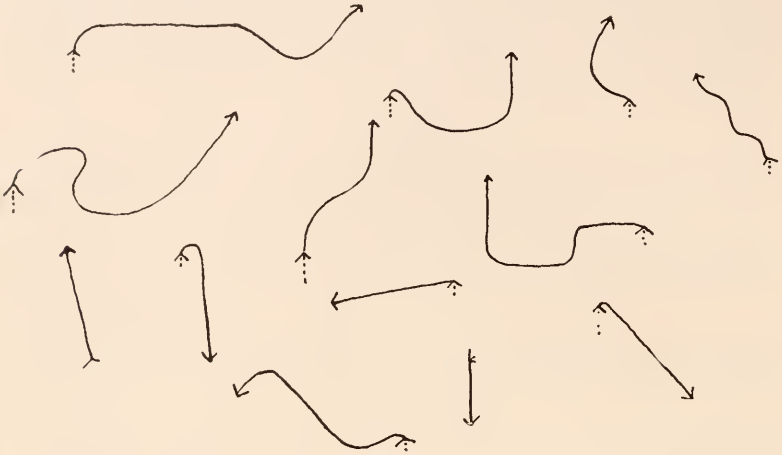


FIG. 2.

The arrows in the figure indicate the movements during the experiment, and the dots the movements of the animals after being placed on the plate but before the commencement of the experiment, *i. e.*, before the plate was covered by the dark box.

One thing is clear. Some of the animals on the dry glass plate and also those placed on a dry wooden plate, in an experiment the results of which will be seen shortly after this, oriented themselves and crawled downward, in spite of the mechanical difficulties due to their shells.

Experiment C.—In the next series, a dry wooden plate was used instead of the dry glass plate, in the same manner as in the above experiment. Of 60 animals used 24 dropped off, 19 or 52 per cent. of the remainder oriented and crawled downward, 6 or 16 per cent. crawled up and 4 crawled horizontally. Positive instead of negative geotropism was dominant, even though the experiments were conducted at the angle of 90°.

Experiment D.—The following series was of the same nature, except that the animals were placed with their heads down, and the time of experimentation was limited to one minute. Of 60 individuals used 33 dropped, 21 or 77 per cent. of those remaining on the plate crawled downward; only 2 oriented negatively and these did not crawl upward; 4 failed to move. As the animals were not compelled to orient themselves in order to move down, the highest percentage of positive geotropism (21 individuals out of 27 or 77 per cent.) was obtained in this case.

That the character of the surface played a part is evident. But some explanation is necessary. When the cover-box was removed, nine individuals which had crawled down as shown by the mucus were found with the head end of their shells directed vertically, or with slight inclination to the vertical, *i. e.*, their shells mechanically "oriented" *upward* by the "greater pull of gravity" on the "heavier posterior region." Those individuals probably could not resist the pull of gravity and keep their shells from falling by their weight, while others could. Four individuals crawled downward not quite vertically (on a diagonal line between vertical and horizontal). It may be possible to explain this as a movement which was resultant of the pull of gravity on their shells and on themselves,—the resultant of a passive tendency to orientation and an internal response to gravitation.

From the above observations, one may explain the habitat of the snails, "which usually live on rocky surfaces which lie between the high-tide mark and one foot below the low-tide mark." The limitation of upward movement is due chiefly to the exhaustion of moisture carried by their "foot" from the sea. They seldom, if ever, crawl on dry rocks much higher than high-tide mark, though they do crawl on rocks clear out of sea-water. They seem to stop crawling when the moisture, which they carry with themselves, is exhausted. This agrees with Mitsukuri's and Haseman's observations.

It is not thinkable that either the dry glass plate, or the dry wooden plate makes it easier for the snails to become oriented and to crawl downward, than the ground-glass plate did. Nevertheless on the former surfaces many animals oriented themselves and crawled downward against the resistance of the heavier weight of their posterior region. If this is true, then the upward and downward movements of the snails can not be explained either by the resistance factor or the weight factor or by the both.

One thing is, of course, possible, that "the quantity and quality of the slime secreted" makes it easier for the animals to crawl downward or upward, as Frandsen thinks in the case of slugs (3, p. 205). The question, however, arises: Why, then, did not the snails crawl upward instead of downward on the the dry glass and wooden plates?

In this respect, Parker and Parshley offer a suggestive explanation in the case of earthworms: "In the earthworm, the response to dryness . . . is apparently the selective extraction of water from the peripheral protoplasm of the worm, a process which is favored by capillarity of dry surfaces over which the worm begins to creep and is probably dependent chiefly upon evaporation from the surface of the worm itself. Under such circumstances, the materials in the peripheral protoplasm of the prostomium must become concentrated and probably initiates stimulation by undergoing some such change as partial coagulation" (15, p. 363).

If so, the explanation must be looked for in physiological conditions, that is, internal, rather than external mechanical factors. As Parker and Parshley explain, the dryness of the surface may cause the partial coagulation of the materials in the peripheral protoplasm of the "foot" as well as of the anterior geo-sensitive region of the snails, which may also result in surface-tension changes and contraction of "muscle elements," and cause the motion of displacement or coalescence of the fluid materials in the protoplasm of the muscle-fibers and internal cells (as Lillie thinks; 7, pp. 252–255), which possess different specific gravities. Under such circumstances, we may assume a disturbance in the peripheral as well as internal cells, especially perhaps in the cells of the geo-sensitive region or the geotropic organs, statocysts, which disturbance may be assumed to stimulate and cause the response of the animals. Whether the animal will go up or down will depend on the other stimuli besides gravity, such as character of surface and degree of dryness which accompany the stimulation of gravity itself. This view quite agrees with Loeb's (9) and Lyon's (11 or 6, pp. 20–21).

4. *The Effects of Light and Surface-film of Sea-water.*

Experiment A.—Experiments were conducted in the aquarium already described, which was closely surrounded by a four-fold black cloth, all over the sides and bottom. The snails were placed on the glass plate about 2.5 cm. below the surface film of sea-water. Part of the glass plate extended above the surface. By this arrangement, it was possible at the same time to test Haseman's conclusion that the upward and downward movements of the animals "are not due directly to either geotropism or phototaxis, but to the action of the film of water" (4, p. 113). The results are given in Table V.

Table V.

Geotropism of Snails in and out of Sea-water in Diffuse Daylight at Different Angles of Inclination of a Glass Plate.

At beginning of experiments each head placed pointing *downward.* Table shows results after one minute.

Temp. of Sea-water.	Angles.	No. of Trials.	No. of Animals.	— Geotropism and + Heliotropism.						— Heliotropism and + Geotropism.				Crawling Horizontally in Sea-water.	
				Crawling up in Air.		Crawling up in Sea-water.		At Surface-Film.		Crawling Downward.		Crawling Diagonally Downward.			
				No.	%	No.	%	No.	%	No	%	No.	%	No.	%
$21\frac{1}{2}^\circ$–$19\frac{1}{2}^\circ$ C.	90°	15	50	14	28	21	42	9	18	0	0	0	0	6	12
$20\frac{1}{2}^\circ$ C.	45°	14	50	7	14	17	34	3	6	7	14	12	24	4	8
$20\frac{1}{2}^\circ$ C.	$11\frac{1}{4}^\circ$	10	50	0	0	2	4	0	0	15	30	27	54	6	12
Total.		39	150	73 or 48.6%						61 or 40.6%				16 or 10.6%	

At the angle of 90°, none were positively geotropic and only six individuals out of 50 crawled horizontally in sea-water (possibly due to the effect of light). At the angles of 45° and $11\frac{3}{4}^\circ$, the number showing "positive geotropism" increased, as would be expected. At the end of one minute, 21 individuals out of 150 (14 at 90° and 7 at 45°), as shown in the first column of negative geotropism, crawled upward clear through the surface-film of sea-water into the air. 12 individuals (9 at 90° and 3 at 45°), as shown in the last column of the same, crawled upward until they reached the surface-film of sea-water, and then crawled horizontally. Was this due "to the action of the film of water," as Haseman claims? The question will be answered after consideration of a few other experiments.

Experiment B.—The next series of experiments was of the same nature as the above, but the animals were placed with their heads upward instead of downward at the beginning. The results given in Table VI. show that the starting position, that is, placing the animal head downward or upward, at the beginning of the experiment, makes a difference in the result.

TABLE VI.

GEOTROPISM OF SNAILS IN AND OUT OF SEA-WATER IN DIFFUSE DAYLIGHT AT DIFFERENT ANGLES OF INCLINATION OF A GLASS PLATE.

At beginning of experiments each head placed pointing *upward.* Table shows results after one minute.

Temp. of Sea-water.	Angles.	No. of Trials.	No. of Animals.	− Geotropism and + Heliotropism.								+ Geotropism and − Heliotropism.				Crawling Horizontally in Sea-water.	
				Crawling Up in Air (No Hesitation at Surface).		Crawling Up in Sea-water (Not yet at Surface).		Hesitated at Surface then Crawled Through into Air.		Hesitated at Surface and Crawled Horizontally.		Crawling Downward.		Crawling Diag. Down.			
				No.	%	No.	%.	No.	%.	No.	%.	No.	%.	No.	%.	No.	%.
19½° C.	90°	14	50	23	46	0	0	6	12	11	22	0	0	0	0	10	20
21¼° C.	45°	13	50	19	38	12	24	3	6	5	10	1	2	8	16	2	4
21½° C.	11¼°	10	50	3	6	24	48	0	0	0	0	8	16	11	22	4	8
Total...........		37	150	106 or 70.6%								28 or 18.6%				16 or 10.6%	

Here, again, eleven individuals at the angle of 90° and five at 45° crawled horizontally beneath the surface-film of sea-water. Besides these, six individuals at the angle of 90° and three at 45° "hesitated" at the surface-film when they reached it, and then crawled upward through the film. This constitutes a puzzle for the surface-film theory. But let us see further.

Experiment C.—Similar experiments were made in direct sunlight. The glass plate in the aquarium was placed at 45°, which made it parallel to the rays of sunlight as nearly as possible. The outside and bottom of the cylindrical glass aquarium were surrounded by black cloth as already stated; and two sections, *a* and *b* (see Fig. 3), separated within by the glass plate, were covered above so that the sunlight penetrated parallel to the glass plate and through the open portion, *c*. So arranged, the intensity and direction of the rays of the sunlight had to affect the reaction of the snails to gravity.

Since the animals were negatively heliotropic, it was expected that most of them would crawl downward in this arrangement. Their heads, therefore, were placed upward at beginning, so that definite movements of orientation could be observed. The results after one minute were as follows: Of 50 animals in 15 trials, 5 or 10 per cent. had crawled upward, all diagonally; 37 or 74 per cent. had crawled downward, 22 of them being well oriented and 15 diagonally; 8 or 16 per cent. crawled horizontally and consequently across the lines of direction both of light and of the effective component of gravity.

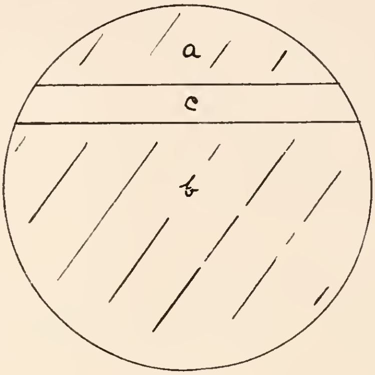


FIG. 3.

The predominance of downward movement was no doubt due to the intensity and direction of the sunlight. This point becomes clearer in the next experiments, but it is clear that the surface-film has nothing to do with such reactions.

Experiment D.—With the same point in mind, other experiments were made in a similar way, but the animals were placed about 1.5 cm. above the surface of the sea-water. In this case 29 individuals out of 50 oriented themselves downward, and crawled in that direction through the surface-film of sea-water into it. Three crawled horizontally at the surface-film instead of going into the sea-water. 18 or 36 per cent. crawled upward.

5. *The Effect of the Surface-film of Sea-water.*

The last of this series was planned in a little different way from the above, entirely excluding light.

As a preliminary test, the snails were placed on the glass plate at the angle of 56¼° in the aquarium, which was half filled with sea-water. When those crawling upward reached the surface-film of sea-water and "hesitated," as Haseman calls it, beneath the film, the whole arrangement was covered with the dark box. Ten seconds later, the box was removed for observation. Nearly all that had "hesitated" at the film, were found to have crawled upward *through* the film.[1] Certain individuals at that time had already crawled upward as high as 3 cm. from the film, some 2 cm., and others were just above the film. This experiment was repeated several times and these results were readily demonstrable. Judging from the results, the animals did not seem to have "hesitated" long, after the light was excluded. The "hesitation" of the snails at the surface of sea-water seems to the writer to be due chiefly to the effect of light instead of to action of the surface-film of sea-water. This point also becomes evident after further consideration.

Quantitative experiments were conducted as indicated above. The results are tabulated in Table VII. and show rather complicated conditions.

Table VII.

Geotropism of Snails at the Different Angles of Inclination of a Glass Plate in and out of Sea-water in Total Darkness.

At beginning of experiment each head placed pointing *downward*. Table shows results after one minute and after another half minute.

Temperature of Sea-water.	Angles.	No. of Trials.	No. of Animals.	— Geotropism									+ Geotropism.	Crawled Horizontally in Sea-water.
				A. After One Minute.						*B*. After Another Half-minute.			After 1 Min.	After 1 Min.
				Clear Out of Sea-water.		Stopped Just Under Surface-film.		Crawling up in Sea-water.		Clear out of Sea-water.	Underneath Surface-film.	Crawling up in Sea-water.		
				No.	%	No.	%	No.	%	No.	No.	No.	No.	No.
22° C.	90°	12	50	26	52	18	36	6	12	18	4	4	0	0
21¼° C.	45°	14	50	23	46	11	22	6	12	14	2	1	3	5
21½° C.	11¼°	11	50	1	2	13	26	5	10	11	8	5	21	10
Total.		37	150	109 or 72%						67			24	15

---

[1] They change their "minds" very rapidly!

"*B*" covers the activity of those animals which were still below the surface at the end of one minute.

After the first minute, twenty-six individuals at the angle of 90°, twenty-three at 45°, and one at 11¼° were found to have crawled upward through the surface-film of sea-water. Eighteen individuals at the angle of 90°, eleven at 45° and thirteen at 11¾° were just beneath the film of sea-water. And after another half-minute, sixteen individuals at the angle of 90° out of the eighteen, which were beneath the film of sea-water at the end of one minute, nine at 45° out of the eleven, ten at 11¾° out of the thirteen, and one at the same angle which was crawling horizontally at the end of one minute, had crawled upward *through* the film of sea-water. This is significant. At the time of second observation, also, at the angle of 90° two individuals out of six, which were crawling upward *in* sea-water at the end of one minute, at 45° five out of six, and at 11¾° one out of ten which were crawling horizontally at the end of one minute, had crawled upward *through* the film. But at the angle of 90° two individuals out of eighteen which were beneath the film at the end of the first period, at 45° two out of eleven, and at 11¾° three out of thirteen, that is, only seven individuals altogether out of 150, *still remained* beneath the film even at the end of the second period. This is decidedly contrary to the surface-film theory. And at the angle of 90° two individuals out of six, which were crawling upward in sea-water at the end of the first period, at 11¾° four out of five, and at the same angle one which crawled downward in the first period, were beneath the film at the end of the second period.

Referring again to the observation of Haseman that "during a falling tide, some snails are crawling down beneath the surface, some with the surface, and some above the surface" (4, p. 120), and which Haseman claims is "due to the action of the film of water," but not "to either geotropism or phototaxis," the writer is inclined to draw the conclusion based on the results of the series of the experiments above, that the movements of the snails in question are not due, directly, if at all, "to the action of the film of water," but to geotropism and heliotropism. In nature, especially in the daytime when the sun is shining, a considerable part would be played by heliotropism, as Mitsukuri already observed.

Furthermore, in the above experiment, even the seven individuals, that still remained beneath the film at the end of the second period, and whose failure to penetrate the film seems to favor the surface-film action theory, may be considered in a different way. The effect of light having been excluded, their failure to emerge might be due to the susceptibility of these individuals to buoyancy when they had to crawl out of sea-water. This must be taken into consideration as has been shown. It might also in combination with the effect of light explain the behavior of those animals that crawled horizontally beneath the film, or that "hesitated" there, as shown in a number of the preceding tables. Whatever may be the explanation and even if one attributes the slight hesitation to the film itself, it is evident that it has very little effect in determining the total behavior of the animals.

6. *The Effect of Chemicals.*

Besides the above experiments, attempts were made to control the negative geotropism of the snails by chemicals, especially by salts and acids. But all were unsuccessful, except possibly an alcohol experiment. Five snails were placed in a finger bowl containing 100 or 150 c.c. sea-water, to which about 10 c.c. of 95 per cent. alcohol were added without stirring. The finger bowl was shaded. The animals crawled upward on the vertical wall of the bowl, but as soon as they reached the upper layers they turned round, and crawled downward. The negative geotropism was thus apparently reversed. But if the alcohol and sea-water were well mixed by stirring, the reversal was not definite and the experiment was not followed further.

IV. Discussion.

That *Littorina littorea* is negatively heliotropic was first shown by Bohn (1), and this fact has been confirmed by the present writer. Morse, however, has published puzzling conclusions from his observations on this species. He states: "During the days of June, they were, as a rule, *negatively* phototactic, and as *night* approached, they became *positively* phototactic. However after July 18, the preponderance of *positive phototataxis during the day* was very noticeable. This period of transition corresponded to the time of change from spring to neap tide, during which the specimens out *on the rocks* were exhibiting a corresponding change in phototaxis, for the water did not reach them; their behavior tallied with the description of Mitsukuri, who showed that when desiccated, periwinkles became positively phototactic, and when wet, turned negatively phototactic" (14, p. 145).[1]

Unfortunately, Morse has given no description of his methods of experimentation. But, judging from his statements, he has entirely overlooked the effect of gravity on the animal, as Mitsukuri did. Was not the supposed "positive phototaxis" "after July 18" really an effect of gravity? *Littorina littorea* does not, in the writer's opinion, crawl upward "on the rocks" on account of positive heliotropism, but on account of negative geotropism and *in spite of* negative heliotropism, which is the unmistakable reaction of the animal to light. Negative heliotropism in the animal is demonstrable even at "night," if the experiment is conducted on a horizontal surface, and if there is any source of light present. The writer believes that Morse's statement that "as night approached, they became positively phototactic" is incorrect. What really happens is this: As the light stimulus diminishes, the gravity stimulus becomes preponderant and the animals are controlled by their negative geotropism.

Frandsen, as already stated, proposes two factors for the determination of geotropism in the slug. The second will be considered first. "*All the conditions being the same,*"[1] he says, "it is this factor (the relative proportions of the length of the anterior and the posterior regions of the animal's body) which 'determines whether the head end will be directed up or down.' If the ratio of the length of anterior to posterior region of body is 2 : 3, or more, and the mucus is of good quality and sufficient quantity, the slug will be positively geotactic. If the ratio is 3 : 5, or less, the animal will usually migrate upward, and the nearer the ratio approaches 1 : 2 the more apt is the slug to respond negatively. . . . All slugs have a natural tendency to move towards the earth. This tendency is masked in the animals which are negatively geotactic on a glass plate by the greater pull of gravity on the disproportionately larger and heavier posterior region of the animal" (3, p. 205).

---

[1] Italics not in the original.

Judging from these statements, the center of gravity must lie somewhere in the posterior region of the animal's body. In other words, the posterior region is *heavier* than the anterior. According to Frandsen, the negative geotropism of the animal "on an inclined glass plate," however, is not due to the heaviness of the posterior region, but to "the relative proportions of the length of the anterior and the posterior regions of the animal's body." In short, Frandsen's idea may be expressed thus: The longer (and heavier?) posterior region being pulled by the constant force of gravity, the slug becomes positive to it at the minimal resistance in favor of the ratio above mentioned; on the other hand, it becomes negative at the maximal in disfavor of the ratio. In the latter sense, the "resistance theory," of course, approaches the "mechanical theory."

As pointed out before, this seems to be fairly supported by the results obtained by the writer, which were given in Tables II., III. and IV. This explanation is, however, focused to a limited group of facts; because the snail becomes negative to gravity on the nearly horizontal surface of a glass plate, where the minimal resistance should be expected. This fact is opposed to Frandsen's conclusion. Furthermore, as has already been shown, many snails on the dry wooden plate at the angle of 90° of inclination oriented themselves downward and crawled in that direction, even though in so doing, they met a great mechanical difficulty on account of the heavier posterior region. If the mechanical theory of Frandsen is true, they were under the most favorable conditions for crawling upward instead of downward. This was not, however, the case. Was this because of the first factor of Frandsen, that is, "the quantity and quality of the slime secreted" (3, p. 205). The second factor in question is by no means separable from the first. Both go together. To make Frandsen's statement clearer, therefore, it may be expressed as follows: "The relative proportions of the length of the anterior and the posterior regions of the animal's body" "being the same, it is this factor," that is, "the quantity and quality of the slime secreted," "which determines whether the head end will be directed up or down." It is fair to examine the table (V.) which is given by Frandsen based on his results. Take the animal No. "13," on which the series of observations, "*a*" and "*b*" were made. The condition of the animal was "active" in series "*a*" and also in series "*b*," that is, it was under the "same" condition. Being the *same* individual, theoretically there was no difference in the "ratio." Nevertheless, Frandsen obtained *different* results in "*a*" from "*b*." Moreover, he shows *different* results in the *same* individual under the *same* condition and in the *same* series of observations, "*a*" or "*b*." This is more striking in the animal No. "23," on which the series of observations, "*a*," "*b*," "*c*" and "*d*," were made under the *same* condition, "good." But he obtained 64.2 per cent. of negative geotropism in series "*a*," 62.5 per cent. in the "*b*," 100 per cent. in the "*c*," and 100 per cent. in the "*d*." In other words, the *same* individual under the *same* condition was, at least, 35.7 per cent. positive in "*a*," 37.4 per cent. in "*b*," and 0 per cent. in "*c*" and "*d*." This kind of variation is also found in the animals Nos. "8," "24," "25" and "27." If so, "all the conditions being the same," it is not entirely the first factor, nor the second, "which 'determines whether the head end will be directed up or down,' " but there must be something else besides these two factors, which makes the response variable. Frandsen does not seem, therefore, to be justified even by his experimental data in his conclusions. And strictly speaking, the so-called mechanical theory of geotropism seems to the writer to be misleading in any case, because it is not a living response to stimulus but a purely mechanical one which would be seen as well in the dead organism, if it could be moved. This is no tropism at all.

In this respect, therefore, the writer is inclined to take Davenport and Perkins's conclusion into consideration, as, at least, one of the factors, that is, "some internal condition of the individual." Without this "internal," or physiological, factor, it is difficult to explain why geotropism varies in the different individuals and also in the same individual at the different angles of inclination of the same support and the same angle of inclination of different supports.

Haseman's conclusions demand close consideration on several points. Haseman states: "Even more interesting is the fact that when, with a vertical surface, a stone, upon which snails were crawling at random, was raised out of the sea, the snails always followed the vanishing film of water even when the vertical surface was rotated through an angle of 180°. In this case, the rotation of the vertical surface would reverse the direction of motion of the film of water and the snails would at once turn around and follow it. But if most of the water was previously removed from the surface of the stone, in order that the film might entirely disappear before the snails (which were crawling downward in the direction of the vanishing film) had reached the lower surface and if, as the film was drying up, the vertical surface was rotated through an angle of 180°, the snails continued to crawl for some time in the direction in which they had started. In other words, the snails crawled upward instead of downward. They continued to crawl thus until the rough surface, food and moisture either deflected or stopped their movements. In the above experiment, the mere turning of the moist but filmless surface through an angle of 180° does not seem adequate to reverse at once the reaction to gravity and light, if either of these have a direct influence on the rhythmical movements of *Littorina*" (4, p. 116).

Certainly this is not a simple matter. In the first phase, Haseman does not state, where "the above experiment" was conducted, what condition of sunlight or diffuse daylight, there was, and if the experiment was in sunlight, in what direction the rays were falling and so on. His description as well as experiment is not at all accurate. Judging from the above description, however, he seems to have conducted the experiment "in nature," and so in sunlight. If so and if the sun were fairly above, it is small wonder that "when the vertical surface was rotated through an angle of 180°," thus rotating the motion of the film of water, "the snails would at once turn around and follow it," since the snails are negative to light, as has been shown. In the case of Haseman where the film was reversed, in which "the vertical surface was rotated through an angle of 180°," and "the snails continued to crawl for some time in the direction (upward) in which they had started," another explanation is possible. The snails being negatively geotropic, the direction and intensity of sunlight were not at the time of this experiment presumably in favor of the reversal of negative geotropism. Therefore, "the snails continued to crawl" "upward instead of downward." This is, of course, reasoned upon the results which were obtained by the writer. At any rate, Haseman seems not to have checked the counteracting forces of gravity and light and consequently all his experiments are unreliable.

One afternoon from four to six o'clock, Dr. Irving A. Field and the writer made special observations of tidal (rising) influence upon the snails at the south shore of Ram Island. The animals were found in large numbers covering a pair of long square beams which formed an inclined railway. These beams presented both vertical and sloping surfaces. In this vicinity there were also rocks and stones of various shapes, their surfaces sloping at many different angles on which numerous snails—oriented with their heads upward—were exposed in *dim* sunlight. When the tide rose higher and higher with no waves and reached to the areas where the animals had been exposed so long that their outer surfaces were completely dried, nearly all of them, if not the entire number, gradually turned head downward and crawled in that direction; some of them moved downward several inches from the original spots, while some others moved about "at random" as if they were seeking food; and still others turned downward when the surface-film of sea-water came in contact with them, while many did so after the surface-film had passed over them to the extent of 0.5 or 1 cm., more or less. If the snails follow "the direction of motion of the film of water," and also, if light has no influence on the rhythmical movements of *Littorina*, as Haseman claims, why did not the snails "crawl upward instead of downward"? Thus considered, it becomes evident that Haseman's observation, or experiment, was not accurate, while Mitsukuri's is, in this respect, confirmed by the writer.

It is very strange to note that Haseman has no notion of the influence of gravity on the snails, although he has observed phenomena which would naturally remind one of it. "When individuals are left high and dry on vertical surfaces during low tide," he says, "they come to rest 'directed upward,' *i. e.*, with their heads toward the sky. This is true for all sides of the stones and is obviously due to the shape of the apertures of the shells which makes it far easier for exposed individuals to cling thus to vertical surfaces" (4, p. 117). How does he know that "the shape of the aperture of the shells" makes it easier to cling to vertical surfaces? Is it not rather true that the natural tendency of negative geotropism of the snails, favored by the *heavier* posterior region of the shells instead of the shape of the aperture, "makes it far easier for exposed individuals to cling thus to vertical surfaces"? This sounds more reasonable and nearer to the fact than Haseman's supposition.

V. Summary and Conclusions.

1. *Littorina littorea* crawls up the vertical (inside) wall of a beaker in a dark aquarium, though the sea-water be better supplied with oxygen at the bottom than at the top. It is negatively geotropic.

2. This snail is negatively heliotropic.

3. The posterior region of the snail has a greater specific gravity than the anterior region.

4. In sea-water, the larger the angle of inclination (to the horizontal) of the surface on which the animals move the larger is the number of negatively geotropic animals; and the smaller the angle of inclination, the larger the number of animals which move downward and are perhaps positively geotropic.

5. In the air, the number of animals showing negative geotropism is always higher than that in the sea-water.

6. On a ground glass plate, the animals are less negatively geotropic than on a plain glass plate.

7. On a dry plain glass plate, a number of individuals oriented positively and crawled downward even at the angle of 90° (vertical) though this never happens on the moist plate. This is more striking on a dry wooden plate.

8. When the animals are placed with their heads down on a dry wooden plate, the highest percentage of positive geotropism is obtained.

9. The snails "hesitate" at the surface-film of sea-water, when light is not excluded.

10. In the direct sunlight, the snails are apparently more positively geotropic than in darkness, due to the fact that they are negatively heliotropic.

11. In the direct sunlight, 58 per cent. of the animals orient themselves head downward and crawl in that direction through the surface-film of sea-water into it.

12. In darkness, the snails, which "hesitated" at the surface-film of sea-water in the daylight, crawl upward *through* the film.

13. From the experimental results which the writer has obtained, he concludes that neither the mechanical theory, nor the pressure theory, nor the resistance theory is adequate to explain the phenomenon of the negative geotropism of *Littorina littorea* but a physiological one, that is, the statocyst or statolith theory. This theory is the more likely since these snails have statoliths (17, pp. 119–120). The writer, however, has no direct evidence, at present, in favor of the statolith theory. He is led to accept it largely by the method of exclusion. Furthermore evidence from many sides based on the experiments of the writer causes him to conclude that the surface-film theory is also not correct.

In conclusion, the writer wishes here to acknowledge his indebtedness to Professors Walter E. Garrey, Ralph S. Lillie, and Elias P. Lyon, for their valuable suggestions and criticism on his experiments at the Marine Biological Laboratory at Woods Hole, Mass., during the summers of 1912 and 1913. His thanks are also due to Professor Frank R. Lillie for the privileges of the Laboratory and to Professor Lyon for criticism and suggestions in the preparation of manuscript.

VI. BIBLIOGRAPHY.

1. **Bohn, Georges.**
'05 Attractions et Oscillations des animaux marins sous l'influence de la lumere. Bull. d. l'Inst. génér. d. Psychol., pp. 171–181.

2. **Davenport, C. B. and Perkins, Helen.**
'97 A Contribution to the Study of Geotaxis in the Higher Animals. Jour. of Physiol., Vol. 22, pp. 99–110.

3. **Frandsen, Peter.**
'01 Studies on the Reactions of Limax maximus to Directive Stimuli. Proc. Am. Acad. Arts and Sci., Vol. 37, pp. 185–227.

4. **Haseman, J. D.**
'11 The Rhythmical Movements of Littorina littorea Synchronous with Ocean Tides. BIOL. BULL., Vol. 21, pp. 113–121.

5. **Jennings, H. S.**
'07 Behavior of the Starfish Asterias forreri de Lorriol. Physiol. Publ. of the University of Calif., Vol. 4, 1907, pp. 53–185.

6. **Kanda, Sakyo.**
'14 On the Geotropism of Paramoecium and Spirostomum. Biol. Bull., Vol. 26, pp. 1–24.

7. **Lillie, Ralph S.**
'12 The Physiological Significance of the Segmented Structure of the Striated Muscle Fiber. Science, N. S., Vol. 36, pp. 247–255.

8. **Loeb, Jacques.**
'88 Die Orientirung der Thiere gegen die Schwerkraft der Erde. (Thierischer Geotropismus.) Sitz.-ber. Würzburg Physiol.-med. Gesell. s. 5–10.

9. **Loeb, Jacques.**
'97 Zur Theorie der physiologischen Licht und Schwerkraftwirkungen. Archiv. f. d. ges. Physiol., Bd. 66, s. 439–466.

10. **Loeb, Jacques.**
'00 Comparative Physiology of the Brain and Comparative Psychology. N. Y., G. P. Putnam's Sons. x + 309 p.

11. **Lyon, E. P.**
'05 On the Theory of Geotropism in Paramoecium. Am. Jour. Physiol., Vol. 14, pp. 421–432.

12. **Mitsukuri, K.**
'01 Negative Phototaxis and Other Properties of Littorina as Factors in Determining its Habitat. Annotationes Zoologicæ Japonensis, Vol. 4, Pt. I., pp. 1–19.

13. **Moore, A. R.**
'10 On the Righting Movements of the Starfish. Biol. Bull., Vol. 19, pp. 235–239.

14. **Morse, Max Withrow.**
'10 Alleged Rhythm in Phototaxis with Ocean Tides. Proc. Soc. Exp. Biol. and Med., Vol. 7, pp. 145–146.

15. **Parker, George H.**
'11 The Mechanism of Locomotion in Gastropods. Jour. Morphology, Vol. 22, pp. 155–170.

16. **Parker, G. H. and Parshley, H. M.**
'11 The Reactions of Earthworms to Dry and to Moist Surfaces. Jour. of Ex. Zool., Vol. 11, pp. 361–363.

17. **Pelseneer, Paul.**
'06 A Treatise on Zoology. Pt. 5. London, Adam and Charles Black, 355 p.

18. **Prentiss, C. W.**
'01 The Otocyst of Decapod Crustacea: its Structure, Development, and Functions. Bull. of Mus. Comp. Zool., Vol. 36, pp. 165–251.

THE GEOTROPISM OF FRESHWATER SNAILS.[1]

SAKYO KANDA.

CONTENTS.

I. Introductory.......... 85
II. Materials.......... 86
III. Experimental.......... 87
    1. Heliotropism of *Physa gyrina* Say.......... 87
    2. Geotropism of *Physa* and Other Species, with the Lung Empty and with the Lung Filled with Air.......... 88
        (*a*) Observations on *Physa*.......... 88
        (*b*) Observations on *Planorbis* and *Limnæa*.......... 89
    3. Geotropism of *Physa* with Lung Empty and Filled with Air, in Presence of Food.......... 92
    4. Geotropism of *Physa* at the Different Angles of Inclination of the Supports in the Air and in Total Darkness.......... 92
        (*a*) Experiments with a Plain Glass Plate.......... 92
        (*b*) Experiments with a Ground Glass Plate.......... 94
    5. Summation of Gravity and Light Stimuli.......... 95
IV. Summary and Conclusion.......... 96
V. Bibliography.......... 97

I. INTRODUCTORY.

Walter (10) and Dawson (2) have investigated the geotropic reactions of *Physa* and other freshwater snails in connection with their respiratory phenomena. However, their observations do not agree on certain points. Walter (10, p. 26) and Dawson (2, p. 93) agree with each other that freshwater snails are negatively geotropic, "when their lungs are empty." The snails being air-breathing forms, it is, of course, necessary for them to crawl up to the surface of water for their air supply, "although their specific gravity is meanwhile gradually increasing through exhaustion of the air," as Walter expresses it. For this upward crawling, the pull of gravity would be expected to act as a "directive force."

Walter and Dawson, however, depart from each other when they come to consider positive geotropism in these snails. The former states that, "after filling the lung with air, they are positively geotropic," so that "they tend to climb down." The latter, on the other hand, states that when the snails "have sufficient air they become indifferent to gravity and crawl in all directions."

---

[1] From the Physiological Laboratory of the University of Minnesota, Minneapolis.

Moreover, they do not agree concerning the behavior of the snails from which "the air supply is cut off." According to Walter, "after reaching the highest point in the flask," which was in an inverted position in water, "and finding themselves unable to renew their supply, their ordinary behavior, to which there were some exceptions, was to let go and drop like dead weights" (10, p. 27). Dawson denies this statement of Walter as follows: "*Physa*, after they have been denied atmospheric air for some time, manifest indifference to the influence of gravity, and scatter over the sides and bottom of the bottle. They have never been observed to let go and drop like dead weights upon being denied atmospheric air" (2, pp. 104, 105).

In this paper an attempt has been made to compare certain experimental results obtained by the writer with those of his predecessors. A comparison is also made with other results obtained by the writer with marine snails.

The experimental work was done in the physiological laboratory of the University of Minnesota, under the direction of Professor E. P. Lyon, during the academic year of 1913–1914, while the writer was holding a Shevlin Fellowship. The writer expresses his appreciation of the interest and suggestions of Professor Lyon throughout the course of the work. To Professor John M. Holzinger, the principal of the State Normal School of Winona, Minn., the writer acknowledges indebtedness for the identification of the forms experimented upon.

II. MATERIALS.

Common freshwater snails, *Physa gyrina* Say, *Planorbis trivolvis*, *Limnæa stagnalis*, and *Limnæa Columella*, were used for the work. The snails were kept in glass aquaria together with green algæ. They seemed to be perfectly healthy, and were observed to grow.

III. Experimental.

To study the behavior of an animal it is always necessary to discriminate the force under consideration as much as possible from other forces which may act simultaneously with it, favorably or antagonistically. Oxygen for pulmonate animals such as *Physa* is, of course, very important. That food is another factor in determining behavior of living animals need hardly be mentioned. Contact stimuli must also be considered. Light is often important. These with gravity are the chief forces which should be borne in mind.

The effect of the force of gravity on *Physa* and others is the problem with which this paper is chiefly concerned. But in considering this the other forces just mentioned must also be considered. Light especially must be taken into account.

1. Heliotropism of Physa gyrina Say.

Walter (10, pp. 23–24) has experimentally shown that *Physa primeana* Tyron and others are generally negatively heliotropic. A series of experiments was made with *Physa gyrina* Say to compare results with the results obtained by Walter. The experiments were conducted as follows: Five selected individuals were placed on a smooth glass plate with their anterior ends facing direct sunlight. The surface of the plate was carefully moistened. During experiments the angle of the rays of sunlight was about 22.5°. The glass plate was horizontally placed in air.

Table I.

Heliotropism of *Physa* on a Horizontal Moist Glass Plate in Air at the Angle of 22.5° of the Rays of Sunlight.

Table shows results after one minute. Feb. 14, 1914, 9:30 A. M. Temperature, 22° C.

No. of Animals.	No. of Trials.	— Heliotropism.		+ Heliotropism.		Horizontally Crawled.	
		No.	%.	No.	%.	No.	%.
1	20	20	100	0	0	0	0
2	20	20	100	0	0	0	0
3	20	20	100	0	0	0	0
4	20	10	50	4	20	6	30
5	20	4	20	16	80	0	0
Total 5	100	74 or	74%	20 or	20%	6 or	6%

The results, given in Table I. confirm Walter's conclusion. However, there are marked individual differences. Number 5, for instance, was exceptionally positive to light, while Numbers 1, 2, and 3 were always negative. Nevertheless, 74 per cent. were negative to light, and only 20 per cent. positive. From these results, the conclusion may be drawn that *Physa gyrina* Say is generally negatively heliotropic.

Dawson (2, pp. 60–61), on the other hand, has observed that darkness interferes with the activity of *Physa*. The writer's observations seem to be in accord with Dawson's. Five of the individuals above mentioned were kept in water under frequent observation one afternoon (from 2.40–4 P. M.) in darkness. Three of them were observed to crawl to the surface and then down again only two or three times; and two of them only once during the period, one 55 minutes and the other 70 minutes after being covered. The rest of the time they did not move at all.

2. *Geotropism of Physa and Other Species, with the Lung Empty and with the Lung Filled with Air.*

As already mentioned, Walter and Dawson disagree on their observations concerning positive geotropism in *Physa* and other species after the air supply is cut off. Neither has, however, furnished the quantitative evidence from which the conclusion was drawn. The writer, therefore, has made some quantitative observations on this point.

(*a*) *Observations on Physa.*—Five selected individuals of *Physa* were placed in a beaker containing about 300 c.c. of water. The beaker, which had vertical sides and a horizontal bottom, was placed as near as possible in optimum daylight. The water which was used for this purpose was taken from the dish in which *Physa* were kept, and was filtered when necessary. The following observations have two aspects, (1) response of *Physa* to gravity, when the lung is empty, and (2) when the lung is full of air. The results are given in Table II.

The negative geotropism of *Physa*, when its lung is empty, is precise and marked. It is impossible to mistake it. It is also evident, on the other hand, that the positive geotropism of the animal after filling the lung, though not as precise as the negative geotropism observed when the lung is empty, is predominant in the majority of cases, that is, in over 90 per cent. Cases of "indifference" to gravity numbered less than 10 per cent. That *Physa* should become positive to gravity after taking in air supply seems to the writer to be quite natural, since the animal is primarily positively geotropic as will be shown later. Moreover, it is worth mention that each individual occasionally showed a peculiar "habit." It crawled up as usual, turned downward when it had reached the surface, and crawled down, making no effort to get air. The number of observations of this phenomenon for each individual is given for reference in Table II. in the second horizontal column and indicated by a star.

TABLE II.

GEOTROPISM OF *Physa* AT THE ANGLE OF 90° OF INCLINATION OF A SUPPORT IN WATER, AFTER ANIMALS HAVE TAKEN AIR IN THE "LUNG-SAC."

Dates.	Temp. of Water.	No. of Trials.	Animal No.	+ Geotropism.		"Indifferent to Gravity" (?)		
				Vertically or almost Vertically Oriented Downward and Crawled.	Diagonally Oriented Downward and Crawled.	Horizontally Oriented and Crawled.	Irregularly Crawled.	Crawled on the Surface-Film of Water.
Dec. 27, 3.10–4.05 P.M. Dec. 28, 3.25–3.50 P.M. Dec. 31, 10.35–11.15 A.M.	19° C.	18	1	7	3	2	1	1
			1*	3	1	0	0	0
		15	2	6	5	0	0	1
			2*	3	0	0	0	0
		11	3	5	1	0	1	0
			3*	4	0	0	0	0
		11	4	8	0	0	0	0
			4*	1	2	0	0	0
		19	5	13	0	0	0	1
			5*	5	0	0	0	0
		74	5	67 or 90.5%		7 or 9.4%		

(*b*) *Observations on Planorbis and Limnæa.*—With single individuals of these genera, the same tests as above were made and still more conclusive results were obtained. Since these snails were comparatively large forms, they were favorable for observation. If they were dislodged they fell to the bottom, provided their lungs were "relatively empty." If the lungs on the contrary were full of air, dislodged snails floated on the surface of water. The same was true in the case of *Physa*. But with this species, as has been already pointed out, it was impossible to exclude light in the experiments because it was inactive in darkness. Consequently the negative heliotropism tended to blur the geotropism. This disadvantage was entirely removed in *Planorbis* and *Limnæa*, because these forms were active even in total darkness.

Observation 1: *Planorbis trivolvis* was put in the 600-c.c. beaker full of water. It either floated or sank, depending on the condition of its lung as above mentioned. If it floated, *i. e.*, was lighter than water, observation on positive geotropism was made; if it sank, *i. e.*, was heavier than water, observation on negative geotropism was made. Either event, therefore, was useful. At about two-minute intervals, the snail was dislodged by a test-tube cleaner, and then was covered by a dark-box. The results are given in Table III.

TABLE III.

Geotropism of *Planorbis trivolvis* on a Vertical Side of a Beaker Full of Water in Total Darkness When it Was Lighter than the Water. Table Shows Results Within Two Minutes.				Geotropism of *Planorbis trivolvis* on a Vertical Side of a Beaker Full of Water in Total Darkness When it Was Heavier than the Water. Table Shows Results After Two Minutes.			
Temp. of Water.	No. of Trials.	+ Geotropism.		Temp. of Water.	No. of Trials.	— Geotropism.	
		No.	%.			No.	%.
19° C.	30	30	100	19° C.	10	10	100

Discussion is hardly needed. A hundred per cent. in both positive and negative geotropisms was obtained. The orientation of positive geotropism in this snail was just as precise as that of negative geotropism.

Observation 2: In the same manner as above, *Limnæa stagnalis* was observed in total darkness. The results are given in Table IV.

TABLE IV.

Geotropism of *Limnæa stagnalis* on a Vertical Side of a Beaker of Water in Total Darkness When it Was Lighter than Water. Table Shows Results After Two Minutes.					Geotropism of *Limnæa stagnalis* on a Vertical Side of a Beaker of Water in Total Darkness When it Was Heavier than Water. Table Shows Results After Two Minutes.			
Temp. of Water.	No. of Trials.	+ Geotropism.		Did Not Move.	Temp. of Water.	No. of Trials.	— Geotropism.	
		No.	%.				No.	%.
19° C.	35	24	68.5	11 or 31.4%	19° C.	25	25	100

As may be seen from the table, the snail did not respond eleven times out of thirty-five trials, when it was lighter than water. This means that it was still floating when the complete two-minute interval was over. The failure of response to gravity in these cases need not be interpreted as "indifference" to gravity, because it was often observed that the snail had opened its air cavity at the time of observation. Evidently it was taking in more air. The geotropic response failed, therefore, because the lung was not full of air. All internal conditions being equal, the snail tends to crawl down, if its lung is full of air. If it crawled downward, it crawled vertically, orienting itself with its anterior end accurately in that direction.

Observation 3: *Limnæa columella*, again, when lighter than water, failed to respond to gravity nearly half the time, as Table V. has shown.

Table V.

Geotropism of *Limnæa columella* on a Vertical Side of a Beaker Full of Water in Total Darkness, When it was *Lighter* than the Water. Table Shows Results After Two Minutes.

Temp. of Water.	No. of Trials.	+ Geotropism.		Did Not Move.
		No.	%	
19° C	35	18	51.4	17 or 48.5%

Only a limited number of observations could be made at a sitting, as the animals ceased to respond at all. This was probably a fatigue effect.

It was observed three or four times that the snail crawled down with its shell in a horizontal position; and two or three times with the anterior end of its shell pointed up. It thus crawled down even against mechanical disadvantage.

The writer was unable to obtain satisfactory observations on this snail's negative geotropism. It crawled up to the surface for air. But when it was subjected to experimentation, it retreated into its shell and did not readily come out.

The writer by the above observations confirms Walter's conclusion regarding the effect of taking air on the geotropism of *Physa* and other species.

3. *Geotropism of Physa with Lung Empty and Filled with Air, in Presence of Food.*

Food being one of the strongest of forces in determining behavior, it was thought advisable to observe its effect on *Physa*. Green algæ were carefully placed on the bottom of the beaker in which there were five selected individuals of *Physa*. It was surprising to find that with food present *Physa* seldom crawled up to the surface for the air supply. After obtaining air moreover most of them crawled down just as precisely as they crawled up. It must also be added that they were not in a starving condition previous to these experiments.

It should be remembered, however, that in this case three forces, that is to say, (1) light, to which *Physa* is negative, (2) gravity, to which it is positive after taking in air, and (3) food, to which it is presumably positive, were here combined. The result of the combination of these three forces is the acceleration of positive geotropism. Negative geotropism, on the other hand, is retarded, even though it is very important for the air supply.

4. *Geotropism of Physa at the Different Angles of Inclination of the Supports in the Air and in Total Darkness.*

Imagining that negative and positive geotropisms of *Physa* and others are due only to respiratory phenomena, Walter claims that "so far as gravity alone is concerned, they should show no response at all" (10, p. 26). According to Dawson, geotropism (negative) is only possible "when their lungs are empty, but when they have sufficient air they become indifferent to gravity and crawl in all directions" (2, p. 93). In a certain sense, therefore, Walter and Dawson agree on this point. This contention will be experimentally examined in this section.

(*a*) *Experiments with a Plain Glass Plate.*—After a few trials it was found that *Physa* was *positively geotropic* even at a slight inclination of a plain glass plate in the air and in total darkness. The following method of experimentation was therefore adopted. The well-moistened glass plate being held slightly inclined, the animal was placed upon it with its head down. When five selected individuals had been so placed, the plate was reversed, and was placed on a "rack" specially made for angle determination. The whole arrangement was covered as soon as possible with a dark box. Curiously enough, darkness seemed not to interfere very much with *Physa's* activity, if the experiment was conducted in the air, though it did interfere in water, as already shown. *Physa* oriented itself in the line of the force of gravity and crawled in that direction. The relative weight of *Physa* is about a thousand times as great in the air as in the water. This probably made a difference in its activity in the air even in total darkness.

The results given in Table VI. show that from the angle of 10¾° of inclination to that of 56¼°, positive geotropism increases as the degree of the angle increases. It should be added that this was not because the lung was full of air. On the contrary, about two thirds of these *positive* animals sank, when they were tested in water. This means that their lungs were empty. Negative geotropism and horizontal crawling, on the other hand, decrease in reverse proportion as the angle of inclination is increased. This was not because the lung was empty. On the contrary, about half of these *negative* animals floated, when they were tested in water.

But there was a limit to the degree of inclination of the support, beyond which *Physa* could not actively move on the plain glass plate on account of the force of gravity. This is significant. At the angle of 67½° positive geotropism suddenly decreases, and negative geotropism and horizontal crawling increase. Gravity, of course, is constant and always exerted vertically. But the effective force exerted on the animals depends upon the inclination of the surface on which the animals crawl. The exertion required to enable the animals to move on a horizontal surface is least; that required on a vertical surface is greatest. At the angle of 67½°, therefore, the effective force of gravity was so great that some individuals of *Physa* could not actively move against it (in air). An apparent increase of negative geotropism, therefore, was the result. This becomes clear, when one considers the failure of the experiments at the angle of 78¾°, which was due to the fact that nearly all the animals passively slid down the plate, mostly with their heads up. The optimum inclination seems to be about 56°. The inference may be made that positive geotropism in *Physa* is an active process, and that negative geotropism, on the other hand, is due largely, though not entirely, to "passive orientation."

The so called mechanical theory of geotropism, however, can not be applied even in the case of negative geotropism. This is obvious when one remembers that *Physa* becomes negative to gravity when "its lung is empty," even though its specific gravity is less than that of water; and positive when its lung is full of air, even though its specific gravity is greater than that of water. The essential factors, therefore, which determine the geotropic orientation, either positive or negative, of *Physa* seem to be internal, that is, physiological ones (cf. 3, 4, 5, 7, 8 and 9 in the bibliography).

TABLE VI.

GEOTROPISM OF *Physa* AT THE DIFFERENT ANGLES OF INCLINATION OF A SMOOTH GLASS PLATE IN THE AIR IN TOTAL DARKNESS.

At beginning of experiments, each head placed upward. Table shows results after one minute.

Temp. of Room.	Angles.	No. of Trials.	No. of Animals.	+ Geotropism.		– Geotropism.		Horizontally Crawled.	
				Oriented and Crawled Downward.		Crawled Upward.			
				No.	%	No.	%	No.	%
20.5° C	67.5°	5	50	29	58	11	22	10	20
20.5° C	56¼°	5	50	47	94	1	2	2	4
20.5° C	45°	5	50	41	82	6	12	3	6
20.5° C	33¼°	5	50	35	70	11	22	4	8
20.5° C	22½°	5	50	31	62	12	24	7	14
20.5° C	11¼°	5	50	30	60	11	22	9	18
Total		30	300	213 or 71%		52 or 19.3%		35 or 11.6%	

From the above results the writer thinks that both Walter and Dawson overlooked the fact that *Physa* is naturally positively geotropic.

(*b*) *Experiments with a Ground-Glass Plate.*—Contact stimuli, as stated, affect the behavior of animals. Supposedly it might affect the geotropism of *Physa*. The same methods were used here as in the above. The results given in Table VII. show a fair agreement with the above supposition.

TABLE VII.

GEOTROPISM OF *Physa* AT THE DIFFERENT ANGLES OF INCLINATION OF A GROUND GLASS PLATE IN AIR IN TOTAL DARKNESS.

At beginning of experiment each head placed upward. Table shows results after one minute.

Temp. of Room.	Angles.	No. of Trials.	No. of Animals.	+ Geotropism.		— Geotropism.		Horizontally Crawled.		Not Moved.	
				Oriented and Crawled Down.		Crawled Up Not Quite Vertical.					
				No.	%.	No.	%.	No.	%.	No.	%.
20° C.	90°	10	50	38	76	0	0	5	10	7	14
	78¾°	10	50	41	82	1	2	5	10	3	6
	67½°	10	50	41	82	3	6	1	2	5	10
	56¼°	10	50	37	74	5	10	2	4	6	12
	45°	5	50	35	70	2	4	5	10	8	16
	33¼°	5	50	32	64	3	6	8	16	7	14
	22½°	5	50	28	56	5	10	4	8	13	26
	11¼°	5	50	24	48	11	22	11	22	4	8
Total.....		60	400	276 or 69%		30 or 7.5%		41 or 10%		53 or 13.5%	

Physa evidently could stick on the ground-glass plate better than on the plain glass plate, as would be expected. Experiments, therefore, could be carried on even at an angle of 90°. Here again, it is noticeable that there is a decrease of positive geotropism at this angle. The optimum inclination in this case is between the angles of 67½° and 78¾°.

5. *Summation of Gravity and Light Stimuli.*

Physa being positive to gravity and negative to light, as already seen, it would be expected to crawl downward even at a small angle of inclination, if it were placed in a strong light. This is just what happened. One morning the rays of sunlight were falling at an angle of about 11¾°. The angle of the rays of sunlight was nearly constant during the experiments. Ten selected individuals were carefully placed with their heads down on a moist plain glass plate. The plate was then reversed and put on the rack whose angle of inclination was 11¾°. They all oriented themselves away from the rays of sunlight, that is, downward, and crawled in that direction. Ten trials were made and there was no exception.

Besides the above, observations on exclusion of the air were attempted, but the results were not satisfactory. Generally speaking however, Dawson's observations seem to be right, although the writer observed one individual "drop" once.

IV. Summary and Conclusion.

1. *Physa gyrina* Say is negatively heliotropic. It becomes sluggish in its activity in darkness, particularly in water.

2. *Physa gyrina* Say, *Planorbis trivolvis*, *Limnœa stagnalis*, and *Limnœa columella*, are negatively geotropic when their lungs are empty; and positively geotropic when their lungs are full of air. *Physa* often comes near the surface and crawls down again without filling its lung with air.

3. *Physa*, when put with green algæ, does not often crawl to the top.

4. At different angles of inclination of a plain glass plate in the air and in total darkness, *Physa* is positively geotropic. There is a certain limit of inclination beyond which the animal can not actively move on account of the force of gravity.

(*a*) Many of the individuals in question are positive to gravity, even though their lungs are empty.

(*b*) The optimum inclination of the plain glass plate on which *Physa* may crawl is an angle of 56¼°.

(*c*) At the angle of 67½°, positive geotropism decreases as negative geotropism and horizontal crawling increase. The negative geotropism is not necessarily the result of lack of oxygen.

(*d*) At the angle of 78¾° no experiments are successful.

5. At different angles of inclination of a ground-glass plate in the air and in total darkness, *Physa* reacts to gravity in a similar manner as though on the plain glass plate, although the limit of inclination is a little higher in the former than in the latter. The optimum inclination of the ground-glass plate is between the angles of 67½° and 78¾°.

6. Contact stimuli seem to interfere slightly with the geotropism of *Physa*.

7. The combination of gravity and light (both the glass support and the rays of light being inclined 11¾° to the horizontal) accelerates positive geotropism of *Physa*.

From the data here given the writer is inclined to draw the conclusion that *Physa* is naturally positively geotropic. It is little wonder, therefore, that *Physa* becomes positive to gravity when its lung is filled with air.

As to the organ of geotropic orientation of *Physa* and other snails, no direct experimental evidence is yet furnished. But according to Cooke (1, p. 196), *Limnœa*, *Planorbis* and *Physa* have statocysts, which are situated near the pedal ganglion, and are probably connected with the cerebral. The statocyst also contains statoliths. The number of the statoliths "varies in different genera and species." There are a hundred in *Limnœa stagnalis* on which the writer has experimented, but about fifty in *Planorbis contratus* and *Physa fontinalis*. Therefore, *Planorbis trivolvis* and *Physa gyrina* Say very probably have statoliths. These statocysts with statoliths may be the organs for geotropic orientation. At any rate, the most probable factors of geotropic orientation, positive or negative, seem to be internal, that is, physiological, and not external.

BIBLIOGRAPHY.

1. **Cooke, A. H.**
   '95 The Cambridge Natural History, Vol. 3, xi–535 p. New York, Macmillan & Co.
2. **Dawson, Jean.**
   '11 The Biology of Physa. Behav. Monog., Vol. 1, No. 4, 111–120 p.
3. **Kanda, Sakyo.**
   '14 On the Geotropism of *Paramecium* and *Spirostomum*. BIOL. BULL., Vol. 26, pp. 1–24.
4. **Kanda, Sakyo.**
   '15 Geotropism in Animals. Am. Jour. Psychol., Vol. 26, pp. 417–427.
5. **Loeb, Jacques.**
   '97 Zur Theorie der physiologischen Licht und Schwerkraftwirkungen. Archiv. f. d. ges. Physiol. Bd. 66, S. 439–466.
6. **Loeb, Jacques.**
   '11 Die Tropismen. Handbuch der Vergleichenden Physiologie, Bd. IV., Erste Häfte, S. 451. Jena, Gustav Fischer.
7. **Lyon, E. P.**
   '05 On the Theory of Geotropism in Paramecium. Am. Jour. Physiol., Vol. 14, pp. 421–432.
8. **Prentiss, C. W.**
   '01 The Otocyst of Decapod Crustacea—Its Structure, Development, and Functions. Bull. of Mus. Comp. Zool., Vol. 36, pp. 165–251.
9. **Verworn, Max.**
   '91 Gleichgewicht und Ostolithenorgan. Archiv. g. d. ges. Physiol., Bd. 50, S. 423–472.
10. **Walter, Herbert E.**
    '06 The Behavior of the Pond Snail Lymnæus elodes Say. Cold Spr. Harb. Monographs, VI, pp. 26–28. The Brooklyn Institute of Arts and Sciences, Brooklyn, N. Y.

After writing this paper the author found three important articles by W. Baunacke, but was not able to take these into consideration in this present paper.

11. **Baunacke, W.**
    '13–'14 Studien zur Frage nach der Statocystenfunktion. Biol. Centralb., Bd. 33, S. 427–452, Bd. 34, S. 371–385, and S. 497–523.

Vol. XXX. *February, 1916.* *No. 2*

BIOLOGICAL BULLETIN

THE MITOCHONDRIA AND OTHER STRUCTURES OBSERVED BY THE TISSUE CULTURE METHOD IN THE MALE GERM CELLS OF CHORTHIPPUS CURTIPENNIS SCUDD.[1]

MARGARET REED LEWIS,

CARNEGIE INSTITUTION.

WM. REES B. ROBERTSON,

UNIVERSITY OF KANSAS.

CONTENTS.

Introduction 99
Literature 100
  Living Material 100
  Fixed Preparations 101
Method 101
Observations 103
  General 103
  Vital Stains 104
  Nucleus 105
  Apical Cell 106
  Primary Spermatogonium 107
  Secondary Spermatogonium (Multiplication Stages) 107
  First Spermatocyte (Growth Stages) 108
  First Spermactocyte Division 108
  Second Spermatocyte (Interkinesis) 109
  Second Spermatocyte Division 109
  Spermatid and Spermatozoön 110
Discussion 112
Conclusion 113

INTRODUCTION.

A study of the chromosomes of *Chorthippus curtipennis* by Robertson (in press) led to the desire to study the mitochondria and other structures of the cytoplasm in order to determine if possible the bearing of the cytoplasmic structures upon the later development.

---

[1] From the Marine Biological Laboratory, Woods Hole, Mass.

It was observed that this material lent itself to the study of the living cell by means of the tissue culture method as described for the chick embryo cell by Lewis, M. R., and Lewis, W. H. ('15) and that not only could the most minute structures of the cell be observed from day to day, but also these structures could be experimented upon as readily as those of the chick embryo. It was decided to study the mitochondria and other cytoplasmic structures of the germ cells of *Chorthippus curtipennis* by means of the tissue culture method.

LITERATURE.

Living Material.—The earliest observations upon the living germ cells of the Arthropods were those of von La Valette St. George ('86) in which he made a careful study of the Nebenkern of the spermatid and described the structure and behavior of that body more completely than many of the later investigators.

Chambers, R. ('15), in his microdissection studies on the germ cell, for which he used the male germ cells of *Disosteira carolina* (grasshopper) and of *Periplaneta americana* (cockroach), gives many interesting observations as to the behavior of the mitochondria during the spermatocyte divisions and he also describes in detail the development of the axial filament of the spermatid and spermatozoön, but apparently Chambers made no effort to trace the cytoplasmic structures of the germ cells throughout their development. The description of the mitochondria during the spermatocyte divisions and the formation of the Nebenkern of the spermatid agrees in general with that found for preparations of *Chorthippus curtipennis* when stained with Janus green. The development of the spermatozoön of *Chorthippus*, however, takes place in quite a different manner from that described by Chambers for the cockroach.

Goldschmidt, R. ('15) states that it was possible to keep the sperm cells of the moth *Samia cecropia* L. alive for three weeks in cultures of hæmolymph and that during this time many follicles finished the process of spermatogenesis. Goldschmidt does not describe the process of spermatogenesis, but merely states that it corresponds with that described for fixed preparations. From these studies upon *Chorthippus* it is quite evident that neither the details of the cellular structure nor their behavior could be carefully studied while the cells remained in the follicle as described by Goldschmidt, but that for this purpose it is necessary to observe isolated cells which lie close to the cover slip.

Fixed Preparation.—The mitochondria and other structures present in various types of fixed material have been studied more or less in detail by numerous observers, with results which depend very largely upon the state of preservation of the material studied. Duesberg, J. ('11) has reviewed this literature so it is unnecessary to repeat it here. In an earlier paper Duesberg, J. ('10) gives a clear and complete description of the behavior of the mitochondria in *Blatta germanica*, which corresponds in all but a few details with that given below for *Chorthippus curtipennis*.

METHOD.

The cultures were prepared in the usual manner (Lewis, M. R., and Lewis, W. H., '15) and all precautions were observed in order to keep them not only chemically clean but also aseptic, except in cases where the period of observation was to extend over only a short time, as for instance when a vital stain was used.

Various different culture media were tried and the one which appeared to be most nearly isotonic with the body fluid of the grasshopper and which also was most favorable for growth was practically Locke's solution *i. e.*, $NaCl$ 0.9 per cent., $CaCl_2$ 0.025, KCl 0.042 per cent., $NaHCO_3$ 0.02 per cent., dextrose 0.25 per cent., peptone 0.2 per cent., but since the observations were made at Woods Hole where running sea water is supplied, the same concentration of salts was obtained by a dilution of the sea water as follows: sea water 30 c.c.+distilled water 50 c.c.+bouillon 20 c.c.+dextrose 0.25 gram+$NaHCO_3$ 0.02 gram. The bouillon was prepared in the same manner as that used for bacteriology, except in this case grasshopper muscle was used in place of beef. A solution of peptone alone can be substituted for the bouillon with rather good results. The culture medium, which is successful, depends largely upon the amount of evaporation which takes place in the technic of the individual observer. This can be determined from the appearance of the preparation itself, for it was found that when the medium was too concentrated the cells formed numerous delicate pseudopodia or flagella and when the medium was too dilute the cells became swollen. The solution which is most nearly isotonic is one in which the cells remain round and flatten out close to the cover slip, or crawl along the cover slip by means of broad, flat pseudopodia.

The grasshopper was opened on the ventral side by means of sterile scissors and the walls pinned down with sterile pins. The testis follicles were then removed aseptically. Each follicle of the testis is made up of a number of cysts, each of which contains a number of cells all in the same stage of development. The apical cell and the primary spermatogonia are at the blind end of the follicle. The cysts which contain the secondary spermatogonia, the first spermatocytes, the synapsis stages, the growth stages, the first spermatocyte division stages, the second spermatocytes, the second spermatocyte divisions, the spermatids, and the spermatozoa are arranged in order back of this towards the open end of the follicle. In order to obtain the cells in the stage desired for observation, a follicle of the testis was placed in a thin drop of the sterile culture medium on a sterile cover slip and, with the aid of a binocular microscope, the wall of the cyst, which contained the cells to be studied, was punctured with a sharp, sterile needle so that the cells of the cyst flowed out into the medium. The excess of the medium was drawn off by means of a capillary pipette and the preparation was then sealed onto a hollow ground slide by means of a vaseline ring. In case stained preparations were to be observed, the vital stain, Janus green or neutral red, was dissolved in the drop of the culture medium in which the follicle was punctured. The cells released from the cyst wall spread out in a thin layer along the cover slip and were then studied by means of the No. 6 ocular and 2 mm. oil immersion lens. A 40-watt Mazda electric light was used for illumination.

Since any stage in the development of the germ cell can be obtained in the above manner, it was not found necessary to watch any one cell over a long period of time, although the cultures remained healthy and dividing cells were found as late as the fourth day.

Tissue cultures of the germ cells in body fluid medium were made as controls, but they were not so useful for the study of the cell structure owing to the fact that the medium is more opaque and that the cells do not spread out in a thin layer close to the cover slip. Also the plasma medium is more difficult to use for experimental purposes owing to the fact that it is easily coagulated.

Observations.

General.—When the cyst wall is broken the cells flow out and become attached to the cover slip. The cells appear to be formed of a clear homogeneous cytoplasm which contains a nucleus and granules. Numerous cells, which contain two or four nuclei and also a correspondingly increased amount of mitochondria, were observed in all stages of development as, for instance, a first spermatocyte with two nuclei and a double amount of mitochondria or a young spermatid with two or four nuclei and also two or four nebenkern. During observations upon one unstained preparation two second spermatocytes whose cytoplasm touched at one point were observed to fuse into a single cell (Figs. 27, 28, 29). The fused cell, which resulted from the two single cells, contained two groups of chromosomes and two groups of mitochondria. In certain stages in the development of the germ cell the granules are scattered throughout the greater part of the cytoplasm (spermatogonium), while in other stages the granules are limited to a definite area (division of spermatocytes). There is no indication of any network structure either of the cytoplasm or of the nucleus. The cells of a cyst remain attached to each other by a long thread-like process, or in some cases by a short thick process, which appears as though the cells had not been completely separated at division, but had remained attached by a band of cytoplasm. Groups of spermatozoa are attached by one end to a crescent-shaped body, while the other end is free and lashes about continuously. Several of these crescent-shaped bodies, each with numerous spermatozoa attached to it, may be seen in one field. The cells may send out broad, flat pseudopodia and crawl along the cover slip, or in media which are too concentrated or to which Janus green has been added, the cells may send out numerous delicate pseudopodia, which appear more like flagella. However, other factors besides the concentration of the medium may influence the size of the pseudopodia, for Goldschmidt (1915) states that the germ cells form flagella in the cultures in hæmolymph and that these flagella can be caused to appear and disappear by a change of temperature.

During mitosis the mitochondria become long, delicate threads and lie around the spindle in such a way as to be easily mistaken for the spindle (Figs. 14 and 22). In none of our preparations were the spindle threads seen and the spindle itself did not show as a cone of material, which had a different light refraction, as it did in the chick. In one preparation the position of the spindle at one pole was outlined (Fig. 16), but even in this case no spindle threads were seen.

The spindle is present however and can be readily shown by means of acetic acid vapor, which destroys the mitochondria and coagulates the cytoplasm sufficiently to show the spindle practically the same as it is shown in figures drawn from fixed material (Figs. 21–26).

Vital Stains.—All stages in the development of the germ cell were studied, not only by means of the living unstained cell, but also by means of preparations stained with Janus green and others stained with neutral red. A few preparations were stained with both Janus green and neutral red. The Janus green stain and also the neutral red stain were dissolved in the culture medium in exceedingly dilute solutions, never more than 1–50,000 parts and frequently as dilute as 1–100,000 parts.

The neutral red stains a large round granule, which is quite different from the mitochondria, not only in size and appearance, but also in behavior (Figs. 1, 22, 36, 37, 39, 41, 46 and 49). This granule has the same reaction to Brilliant cresylblue 2 b. as that of the granule described by Lewis and Lewis ('15) in connection with the "vacuole" and agrees in a few details with the "beta globule" described by Coghill ('15). In a few cases the granule reacts with the neutral red stain in the same manner as does the neutral red granule, which Renaut, J. ('04) and Dubreuil, G. ('13) describe in connection with the connective tissue development. Owing to the fact that the literature does not furnish a satisfactory term for this granule, it will be called simply the neutral red granule in the following observations. Duesberg ('10) in certain figures, as for instance those of the spermatid, shows a granule in the same position as that of the neutral red granule in our preparations, and, although the granule is stained like the mitochondria with Benda's stain, Duesberg states that it is in all probability not mitochondria. In preparations stained with Janus green this granule remains unstained. The somatic cells, which form the wall of the follicle and also the apical cell (Figs. 1 and 2), are full of these bright red granules when the preparation is stained with neutral red, but the germ cells contain only a very few neutral red granules.

With Janus green stain however, the germ cells are shown to contain abundant mitochondria in the form of granular threads or of small rod shaped granules. After the preparation has been stained for a short time the granules coalesce into larger globules (Figs. 4 and 5) and finally they disappear in the cytoplasm. Long, thread-like mitochondria rapidly break into granules when stained with Janus green (Figs. 13–20).

Neither of the above stains showed the spindle threads during mitosis. There was no appearance seen at any time during observation upon either the unstained cell or the cell stained by means of the above vital stains, which could lead one to conclude that the mitochondria are formed from any material at the expense of the nucleus as Wassilieff ('07) and his followers contend.

Nucleus.—The various changes which the nucleus undergoes during the so-called resting stage and during division can be clearly observed throughout the development of the germ cells from spermatogonia to spermatozoa. In the resting nucleus of any stage chromatin threads were observed. In the nucleus of the spermatogonium these chromatin threads or spiremes seem to fill the nuclear space like so many sacs (Fig. 1). When the preparation was stained with neutral red, the walls of these sacs became faintly pink and so revealed the boundaries of the chromosomes. During the telophase, and in a few cases, in the late anaphase of the spermatogonium, the spermatocyte and the spermatid, the chromosomes have a granular structure (Figs. 4, 10, 20). These granules appear to be uniform in size and it might prove possible to count the number of granules which compose a given chromosome. The importance of these granules in "crossing over" phenomena may appear later.

The number and also the characteristics of the chromosomes observed in the living cells correspond with what was found in the fixed preparations. In the first spermatocyte the chromosomes were as follows: Five "short rod" tetrads, three large "compound ring" tetrads, derived from the three pair of compound V chromosomes of the spermatogonium and the rod-like sex chromosomes. In this genus, *Chorthippus* formerly known as *stenobothrus*, there is a peculiar compounding of six pairs of rod chromosomes to form the three pairs of V's that are characteristic so far as is known of all the species of the genus (Meek, '11, '12; Gerard, '09; Davis, '08).

This peculiar process by which the three pairs of compound V chromosomes are formed was first observed by Robertson (in press). The subsequent behavior of these compound chromosomes was the same in the living cell as has been described from the fixed preparations and the five rods, three V's and the sex chromosomes (present only in one of the two daughter cells, which result from the first spermatocyte division) were easily identified in the second spermatocytes and in the spermatids.

The Apical Cell.—The apical cell lies in the blind tip of the follicle surrounded by primary spermatogonial cells (Figs. 1, 3). It is a round cell, which contains a more or less oval nucleus, and is attached to the walls of the follicle by several thick cell processes. The apical cell contains both mitochondria and neutral red granules (Figs. 1 and 3). The former are fewer in number than the latter and uniformly small in size (Fig. 3). They are arranged as granular threads mostly in a layer around the nucleus. The neutral red granules are considerably larger than the mitochondria and are scattered throughout the cytoplasm and in the cell processes. When a preparation is stained with neutral red, these granules in the apical cell and also in all of its processes rapidly take up the stain, so that the apical cell becomes quite red in appearance. While the few granules in the spermatogonia which surround the apical cell take up the stain only after a long time and then only in a few scattered granules so that the spermatogonia appear practically colorless. The somatic cells (Fig. 1), which form the wall of the follicle, have abundant neutral red granules and these stain with neutral red in much the same manner as did those of the apical cell. This striking resemblance of the apical cell to the somatic cells in contrast to the germ cells suggests the possibility that the apical cell may be more closely related to the somatic cells than to the germ cells.

Primary Spermatogonia.—In the primray spermatogonia both the mitochondria and the neutral red granules can be identified in the unstained cell. In the resting cell the mitochondria appear as delicate granular threads and at this time these threads seem to radiate from the distal pole of the cell (*i. e.*, the region of the last connection with its sister cell at mitosis). The mitochondria of the primary spermatogonia stain less intensely with Janus green than does that of cells in a later stage of development and when stained with Janus green the delicate threads become rapidly distorted and appear as granules. The neutral red granules, from 4 to 10 or 12 in number, are larger, more or less round and much more refractive than the mitochondria granules. These neutral red granules, so far as was seen, did not seem to be located in any definite region of the cell, but were scattered through the cytoplasm.

Secondary Spermatogonia (*Period of Multiplication*).—The secondary spermatogonial cells are smaller than the primary spermatogonia and the mitochondria are usually in the form of fine, granular threads scattered from the distal end of the cell towards the nucleus. As the cell approaches the resting condition the mitochondria become more uniformly scattered throughout the cytoplasm. In a few observations the mitochondria appeared to be absent from a region at the extreme distal pole of the cell, possibly the mitosome (*i. e.*, the remains of the spindle, Figs. 2, 4, 6).

During mitosis the mitochondria arrange themselves as long threads close to the spindle and frequently they have the appearance of abnormally thick spindle threads. During the constriction of the cytoplasm at late anaphase the mitochondria threads again become granular and at telophase the mitochondria are separated into two practically equal amounts, one of which passes into each daughter cell and from this mass the mitochondria migrate towards and partly around the nucleus (Fig. 4), as can be seen in the first spermatocyte.

First Spermatocyte (*Growth Period*).—As the cell grows in size the amount of mitochondria appears to increase correspondingly and certainly the mitochondria of the spermatocyte stain much more intensely with Janus green than do those of the spermatogonium. The neutral red granules are few in number and scattered throughout the cytoplasm.

In the later growth period the mitochondria become grouped into two, or in a few cases, possibly more masses (Figs. 7 and 8 and 11). Unfortunately the significance of this was not comprehended, but it is without doubt closely allied with some change in the cell itself, as the massed arrangement of the mitochondria is quite characteristic of the synapsis stages. There was no evidence that the mitochondria granules paired during synapsis, although the pairing of the chromosomes was clearly seen.

First Spermatocyte Division.—During the prophase and early metaphase of the first spermatocyte, the mitochondria migrate away from the two masses of mitochondria granules and elongate towards the two poles of the spindle (Fig. 12), so that when the chromosomes are arranged on the spindle plate, the mitochondria appear as threads which more or less closely surround the spindle. As the chromosomes move apart, the mitochondria become drawn out into straight, even threads closely attached to the spindle between the two groups of chromosomes (Figs. 13, 14 and 15). Since they are much longer and more refractive at this stage, they may be easily mistaken for the spindle fibers and some cytologists have stated that the mitochondria in the germ cell are but the remains of the spindle fibers. A simple experiment shows that this is not the case in *Chorthippus curtipennis*. Figs. 21, 22 and 23 were made from the living cells in the cultures and then an opening was made in the vaseline ring, which supported the cover slip and a drop of glacial acetic acid was placed on the slide near to the opening so that the fumes from the acid passed through the opening and acted upon the preparation. The mitochondria were destroyed at once and became lost within the coagulum of the cytoplasm, and, where previously no spindle could be seen, there now appears a typical spindle (Figs. 24 and 25). Fig. 26 shows the remains of the spindle fibers near the periphery of each daughter cell, but the mitochondria, which previously lay along these threads (Fig. 23) and scattered towards the nucleus, have now disappeared.

During the anaphase of the living cell the daughter chromosomes move to the extreme proximal pole of the cell and when the cytoplasm constricts, the continuous mitochondria threads again become granular and from the ends of these threads the mitochondria granules migrate away towards the two groups of chromosomes (Fig. 22). In some instances the entire mitochondria thread appeared to pass over to one daughter cell, but usually the division of the mitochondria took place by means of the migration of the granules into each daughter cell, so that each daughter cell received about the same amount of mitochondria.

When the preparation was stained with Janus green the mitotic division continues, provided it was started before the stain acted upon the cell (Figs. 17–20), but after the division was finished, the cell did not pass through the second spermatocyte division as the unstained cells did. The mitochondria threads become granular and do not usually extend from chromosome group to chromosome group, but more frequently lie in a broad band around the equator of the spindle (Figs. 16, 17, 18 and 19). The ends of the granular threads swell up and become varicose before the granules migrate away (Figs. 19 and 20).

Second Spermatocyte (*Interkinesis*).—After the mitochrondria have migrated into the daughter cells the cytoplasmic bridge between the two cells remains and elongates. The cells may sometimes remain thus attached by a long process during the semi-resting condition (interkinesis), which may continue for an hour or even for several hours before the cells prepare for the second spermatocyte division. The second spermatocyte has a characteristic appearance and is easily recognized. The chromosomes remain and it can be seen that they are no longer tetrads as they were in the first spermatocyte (Fig. 30). The mitochondria are gathered into an irregular body at one side of the cell and from this body the mitochondria granules spread out somewhat toward the nucleus (Fig. 30).

Second Spermatocyte Division.—The behavior of the mitochondria during the second spermatocyte division is practically the same as that during the first spermatocyte division. In this case however there is usually only one mitochondrial mass (although it may divide into two in some cases) and this mass lies at one side midway between the two poles of the spindle (Fig. 30). The mitochondria migrate away from this granular mass and elongate towards the two poles of the spindle. Usually the elongated threads spread out around the spindle, but in a few cases they have been seen to remain mostly on the side of the spindle where the mitochondrial body was. The threads become homogeneous and appear to be continuous threads stretched between the two groups of chromosomes. When the cytoplasm constricts during anaphase the granules migrate into the daughter cells, but they do not usually scatter around the nucleus. A few neutral red granules are present in the cytoplasm throughout the division and can be seen in the cytoplasm on each side of the mitochondrial body in the spermatid (Figs. 36 and 37). When division is completed the mitochondria granules become contracted into a compact, spherical granular body, the nebenkern, and a few neutral red granules are irregularly scattered on each side of the nebenkern. It is interesting to note that after acetic acid vapor, a round body can still be distinguishable in the place of the nebenkern, so that either the mitochondria are at this stage more resistant to acetic acid, or else there is some other body present in the nebenkern.

In a few cases the daughter cells seemed to be unequal in size. It has not been determined whether the inequality in size of the daughter cells is due to the presence of the sex chromosome in the larger cell. Davis ('08) and Gerard ('09) in their figures for this genus each show one of the daughter cells, which result from the second spermatocyte division, larger than the other, but they do not call attention to this point.

The Spermatid and Spermatozoön.—The development of the spermatid into the spermatozoön is by no means a simple process and, even after prolonged observation, the significance of the various steps is not clear (Figs. 38–51).

The neutral red granules do not play an active part, but remain about the same in the cytoplasm until with the growth of the tail the cytoplasm becomes small in quantity and they disappear. They were not observed in the more adult spermatozoön except in cases where some abnormal factor caused the cytoplasm along the tail of the spermatozoön to become clumped into nodes, and, in this case, one or more neutral red granules were present in each node (Fig. 49). In the young spermatid the mitochondria are in the form of the granular nebenkern (Fig. 38), which later becomes a clear, homogeneous, spherical body close to the nucleus. No trace of granules can be seen either in the unstained cell or in those stained with Janus green. Within a short period of time (½ hour) certain delicate threads appear within the clear nebenkern (Figs. 39 and 40). These threads may be concentric or otherwise coiled and may represent either the rows of granules under pressure, or they may be the edges of the membrane, which separates the nebenkern into two half spheres, seen at different levels, for very shortly after the threads appear, the nebenkern slides apart into two half spheres and at once becomes granular again (Figs. 41, 42, 43).

The behavior of the centrosome and the formation of the axial filament, which has been described from fixed material, was seen only in one case, and in that case it was not possible to ascertain just what connection there is between the division of the nebenkern and the formation of the axial filament. The centrosome was seen as a clear paired body near the nucleus, but at a distance from the mitochondrial body (Fig. 38). Later the centrosome occupies a position at the posterior pole of the nucleus near the Nebenkern. After the division of the Nebenkern it was seen as a small opaque body close to the nucleus (Fig. 44). The body is double and, although small, it increases in size with the growth of the tail and later becomes the middle piece (Figs. 46, 49, 50 and 51). From this body the axial filament probably is given off and later grows out into an extension of the cytoplasm.

The mitochondrial bodies, or the two half spheres of the nebenkern, now elongate as granular sacs (Figs. 45 and 46). The wider end is towards the nucleus and the sacs gradually taper off until they become only a few granules at the other end (Fig. 46). As the tail grows out these bodies grow out one on each side of the axial filament. The sacs do not increase in size, but simply spread out along the axial filament and become crowded into the narrow layer of cytoplasm along the tail, so that as the tail elongates the granules form two irregular granular strands, which later fuse into two continuous threads of even width extending from the centrosome body or middle piece to almost the end of the tail (Figs. 49, 50 and 51).

Duesberg ('10) describes a small body composed of mitochondria at the tip of the head, but in this form such a body was not seen either in the living spermatozoön or in those stained with Janus green.

Unfortunately it was impossible to study this material in all its details during the short time at Woods Hole and there are many interesting points to be followed out, which it is hoped may be continued in later studies upon another species of grasshopper by Robertson.

Discussion.

The above observations show that there are present in the male germ cell from its earliest appearance throughout its development certain granules which correspond to the mitochondria of somatic cells. The mitochondria behave in a definite and characteristic manner during the development of the male germ cell and assume a shape and position characteristic for each stage in the development. The mitochondria of the germ cell become slightly blackened with osmic acid, are destroyed by acetic acid and stained by Janus green and the usual stains for mitochondria in fixed material. That they are not the remains of spindle fibers has been clearly shown by means of acetic acid, which destroys the mitochondria and causes the spindle fibers to appear. On the other hand it does not seem possible that bodies, which have to do only with the metabolic activities of the cell should necessarily undergo such an exact behavior as is shown for instance by the division of the nebenkern into equal parts and the development of these two sacs of mitochondria into the two long threads of mitochondria in the spermatozoön. Neither does the behavior of the mitochondria seem to be entirely dependent on changes within the cell, as for instance during the division of the cell or the growth of the tail, where it might appear that the elongation, and at the same time narrowing of the tail, might force the mitochondria granules to assume the position of two continuous threads, for in some cases where the tail does not develop as normally but the axial filament grows out within the cell, the mitochondria develop as normally in connection with the axial filament and form the same long threads, although in this case these threads are wound round and round within the cell (Figs. 47 and 48).

The origin of the mitochondria is still unsolved. They certainly do not arise in the male germ cells, since they are already present in the earliest germ cell. There is no evidence in the above observations that the mitochondria are formed at the expense of any nuclear material. Also there is no evidence that the mitochondria of the male germ cell of *Chorthippus curtipennis* can have any influence upon inheritance, as was suggested by Meves ('13) in his work on *Ascaris*, unless it can be shown that the tail as well as the nucleus of the spermatozoön enters the egg.

Conclusion.

The mitochondria as well as the neutral red granules are present in the primary spermatogonium of *Chorthippus curtipennis* and, while the neutral red granules appear to have no definite behavior, the mitochondria do behave in a characteristic manner throughout the development of the male germ cells. By means of the tissue culture method the mitochondria can be seen to be present as small, delicate granules in the primary spermatogonium. They increase in amount during the growth stage and arrange themselves along the spindle in a definite manner during the spermatocyte division. They form the nebenkern of the spermatid and from this develop into two equal homogeneous threads in the tail of the spermatozoön.

Woods Hole, Mass.,
September, 1915.

LITERATURE LIST.

Chambers, R.
'15 Microdissection Studies on the Germ Cell. Science, N. S., Vol. XLI., No. 1051.

Coghill, G. E.
'15 Intracellular Digestion and Assimilation in Amphibian Embryos. Science, N. S., Vol. XLII., No. 1080.

Davis, H. S.
'08 Spermatogenesis in Acrididæ and Locustidæ. Bull. Mus. Comp. Zoöl. Harvard College, Vol. LIII, No. 2, p. 59.

Dubreuil, G.
'13 Le Chondriome, etc. Arch. d. Anat. Micr., Tom. 15, p. 53.
Duesberg, F.
'10 Nouvelles recherches sur l'appareil mitochondrial des Cellules seminales. Arch. f. Zellf., Bd. 6.
'11. Plastosomen "apparato reticolare interno" und Chromidial apparat. Ergeb. d. Anat. u. Entwickgeschichte, Bd. XX.
Gerard, P.
'09 Recherches sur la spermatogénèse chez Stenobothrus biguttulus. Arch. Biol., tom. 24, p. 543.
Goldschmidt, R.
'15 Some Experiments on Spermatogenesis in Vitro. Proc. Nat. Acad. of Science, Vol. I., p. 220.
La Valette, St. George, von.
'86 Spermatologische Beiträge Zweite Mitt. Arch. f. mikr. Anat., Bd. XXVII.
Lewis, M. R., and Lewis, W. H.
'15 Mitochondria and Other Cytoplasmic Structures in Tissue Cultures. Amer. Jour. Anat., Vol. XVII., p. 339.
Meek, C. F. U.
'11 The Spermatogenesis of Stenobothrus viridulus with Special Reference to the Heterotropic Chromosome as a Sex-determinant in Grasshoppers. Jour. Linnean Soc. (London), Zoöl., Vol. XXXII., No. 208, p. 20.
'12a A Metrical Analysis of Chromosome Complexes, etc. Philos. Trans. Roy. Soc. London, B, Vol. 203, p. 84.
'12b The Correlation of Somatic Characters and Chromatin Rod, etc. Jour. Linnean Soc. (London), Zoöl., Vol. XXXII., p. 107.
Meves, F.
'13 Über die Beteiligung der Plastochondrian an der Befruchtung des Eies von Ascaris megalocephala. Arch. f. mikr. Anat., Bd. 76.
Renaut, J.
'04 Les cellules Fixes des Tendons de la queque du jeune Rat sont Toutes des Cellules Connectives Rhagiocrines. C. R. de la Soc. de Biol., 1904, p. 1067.
Robertson, W. R. B.
Chromosome Studies. I. Taxonomic Relationships shown in the Chromosomes of Tettigidæ and Acrididæ: V-shaped Chromosomes and their Significance in Acrididæ, Locustidæ, and Gryllidæ: Chromosomes and Variation. (In press, Jour. Morph.)
Wassilieff, A.
'07 Die Spermatogenese von Blatta germanica. Arch. f. mikr. Anat., Bd. LXX.

EXPLANATION OF PLATES.

J. G. = Janus green stain.
N. R. = neutral red stain.
Mitochondria = black-inked granules and threads.
Neutral red granules = gray granules outlined with black ink.
Centrosome = clear round granule about same size as neutral red granule.

Plate I.

Fig. 1. N. R. Blind end of follicle. Apical cell surrounded by primary spermatogonia. The somatic cells form the boundary of the follicle. Round neutral red granules in the apical cell and also in the somatic cells. Leitz Binoc. 4b × 1/12 lens.

Fig. 2. Unstained cell. Primary spermatogonium. Mitochondria are granular threads. Clear region at upper side of the cell is probably the mitosome (spindle remains). Leitz Binoc. 4b × 1/12 lens.

Fig. 3. J. G. and N. R. Apical cell. Mitochondria are small black granules and the neutral red are the larger gray granules. Zeiss 6 oc. 2 mm. lens.

Fig. 4. J. G. Two secondary spermatogonia still attached together. Chromosomes granular. Telophase. Mitochondria are not in nucleus but around it. Leitz Binoc. 4b × 1/12 lens.

Fig. 5. Same cell as Fig. 4. Globules of blue which form after the mitochondria have lost their stain.

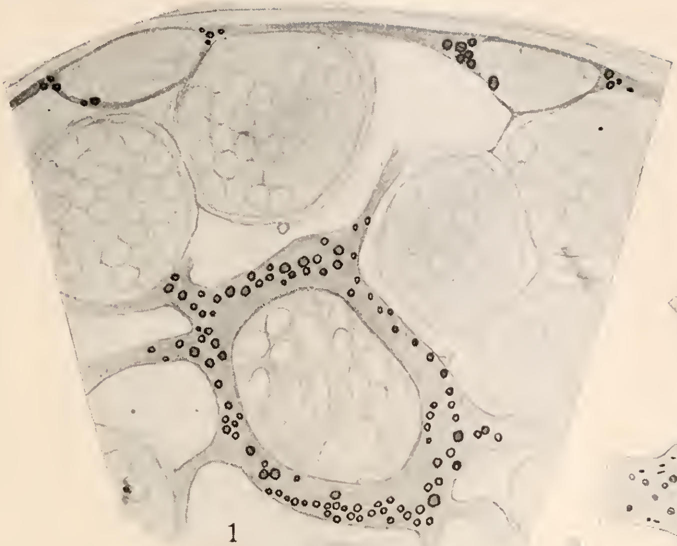
Fig. 6. J. G. Secondary spermatogonium.

Fig. 7. J. G. First spermatocyte, centrosome divided. Sex chromosome. Early prophase.

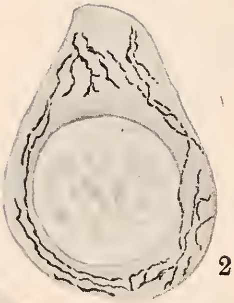
Fig. 8. J. G. First spermatocyte. Diplotene nucleus stage. Centrosomes. Two masses of mitochondria, one on upper surface of cell and one on the lower. Leitz Binoc. 4b × 1/12 lens.

Fig. 9. Unstained. First spermatocyte 'bouquet-stage' synapsis. Zeiss 6 oc. 2 mm. lens.

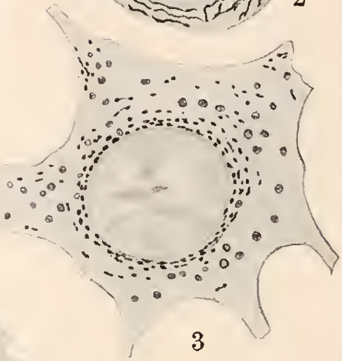
Fig. 10. J. G. Spermatogonium, telophase. Each chromosome is in a separate vesicle and is granular. Nuclear wall reforming from chromosome vesicles. Leitz Binoc. 4b × 1/12 lens.



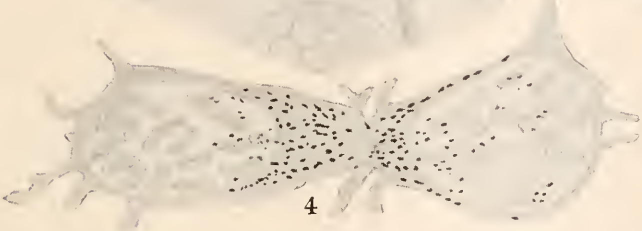
1



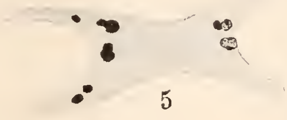
2



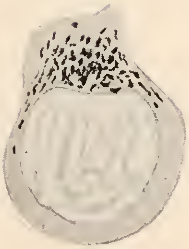
3



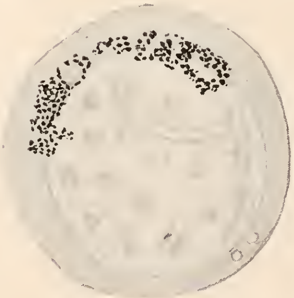
4



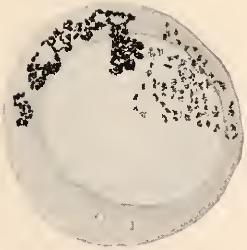
5



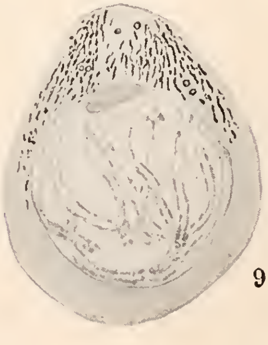
6



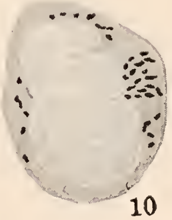
7



8



9



10

M. R. LEWIS AND W. R. B. ROBERTSON.

PLATE II.

FIG. 11. J. G. First spermatocyte, late prophase. Nuclear wall still intact, two masses of mitochondria.

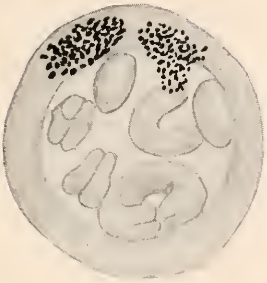
FIG. 12. Unstained. First spermatocyte metaphase. Mitochondria threads have migrated towards the poles of the spindle. Zeiss 8 oc. 2 mm. lens.

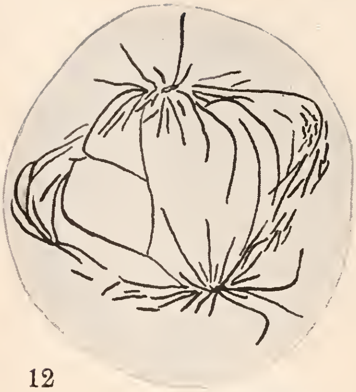
FIGS. 13, 14, 15. Unstained. First spermatocyte division. Same cell at 9:30 A.M. (Fig. 13), 9:45 A.M. (Fig. 14), and 10:10 A.M. (Fig. 15). Mitochondria are long threads close to the spindle fibers. Zeiss 4 oc. 2 mm. lens.

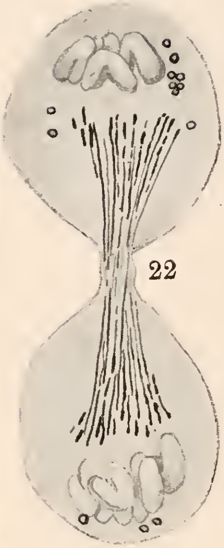
FIG. 16. Unstained. Mitochondria are granular threads around the spindle. Those in the middle of the spindle occur underneath. The sex chromosome at one pole. Spindle outlined at one pole. Leitz Binoc. 4b × 1/12 lens.

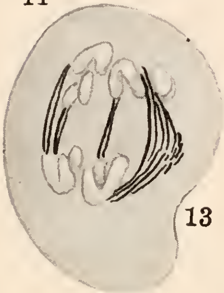
FIGS. 17, 18, 19. J. G. First spermatocyte division in the same cell. Fig. 17 at 9:40 A.M., Fig. 18 at 9:50 A.M., and Fig. 19 at 10:45 A.M. Zeiss 6 oc. 2 mm. lens.

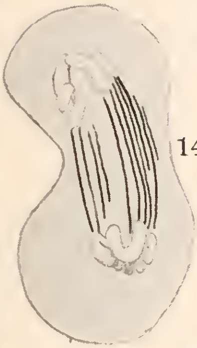
FIG. 20. J. G. End of first spermatocyte division. Mitochondria migrated into two daughter cells. Chromosomes granular. Leitz Binoc. 4b × 1/12 lens.

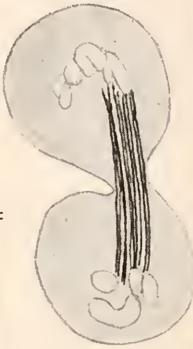

11

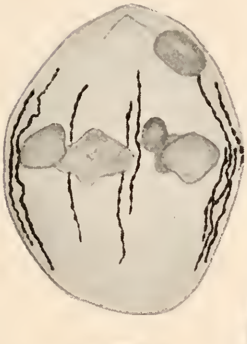

12

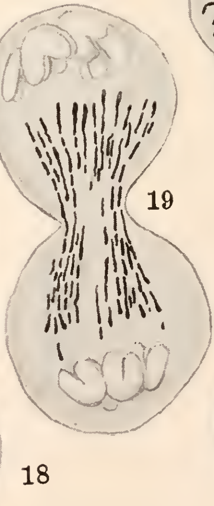

22

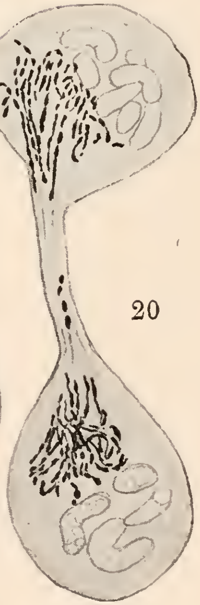

13

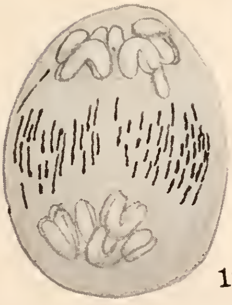

14

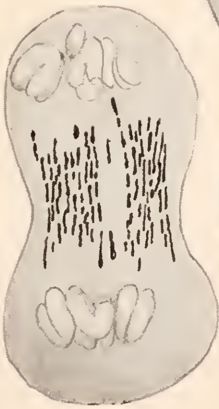

15


16


19


20


17


18

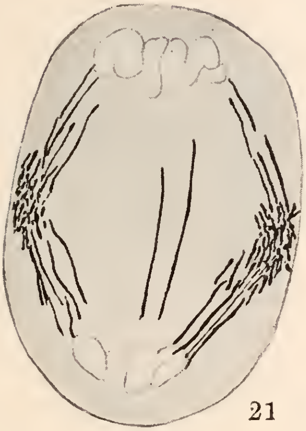
M. R. LEWIS AND W. R. B. ROBERTSON.

Plate III.

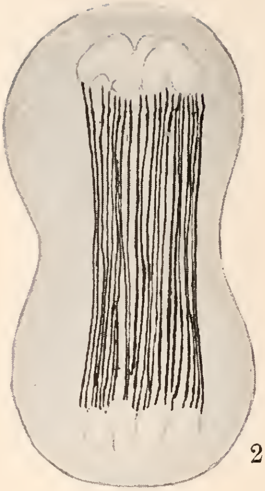
Figs. 21, 22, 23. Unstained. Division stages drawn to show mitochondria before the action of acetic acid. Zeiss 8 oc. 2 mm. lens.

Figs. 24, 25, 26. Same cells after acetic acid fumes. Mitochondria have disappeared and the cytoplasm is coagulated. Spindle fibers can now be seen.

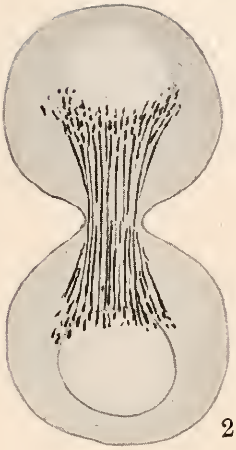
Figs. 27, 28, 29. Unstained. The fusion of two cells to form a single double cell with two groups of chromosomes and two masses of mitochondria. Leitz Binoc. 4b × 1/12 lens. Chromosomes not all shown.



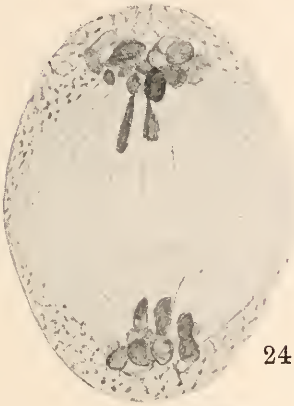
21



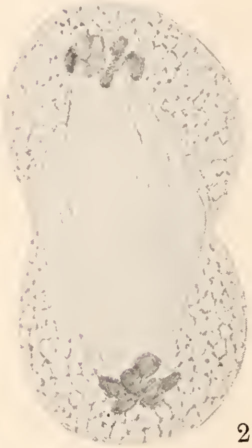
22



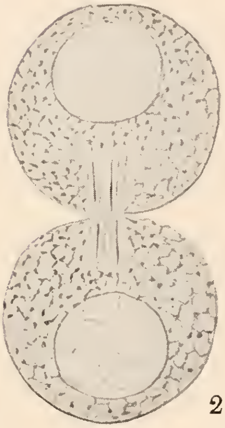
23



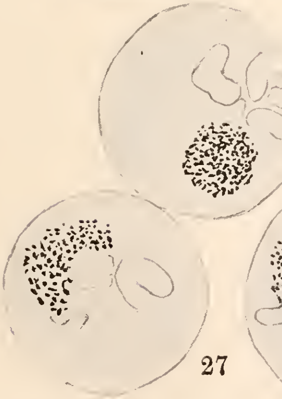
24



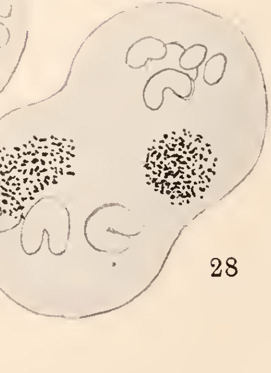
25



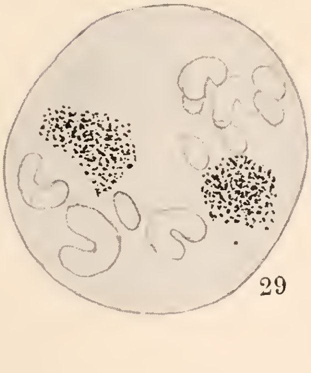
26



27



28



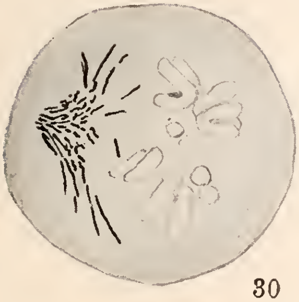
29

PLATE IV.

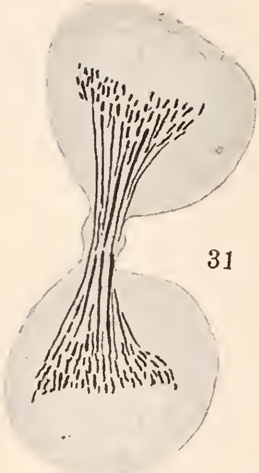
FIG. 30. Unstained. Second spermatocyte. Single mass of mitochondria from which granules are elongating toward each pole of the spindle. Zeiss 6 oc. 2 mm. lens.

FIGS. 31–35. Unstained. Different stages of a cell in the second spermatocyte division. Fig. 31 at 10:40 A.M.; Fig. 32 at 11:20 A.M.; Fig. 33 at 12:30 P.M.; Fig. 34 at 1 P.M.; and Fig. 35 at 2 P.M. The mitochondria form the nebenkern.

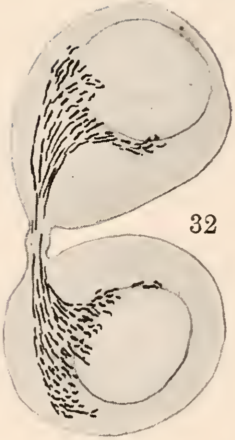
FIGS. 36, 37. N. R. Neutral red granules present during the second spermatocyte division. Zeiss 6 oc. 2 mm. lens.



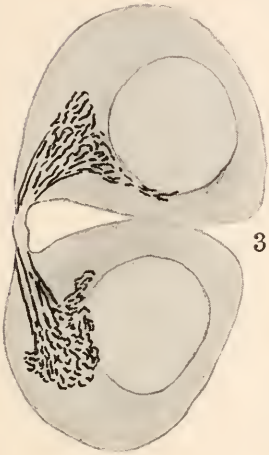
30



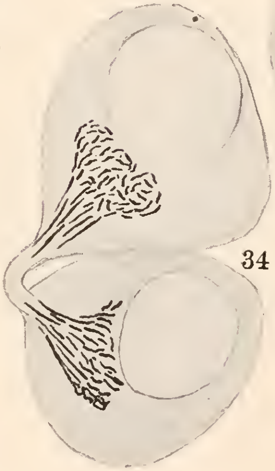
31



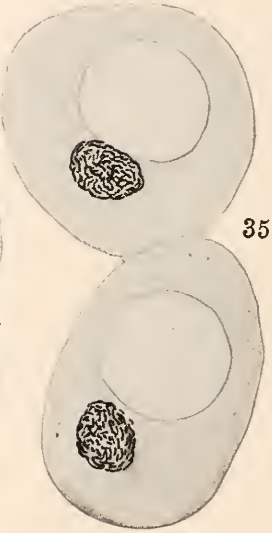
32



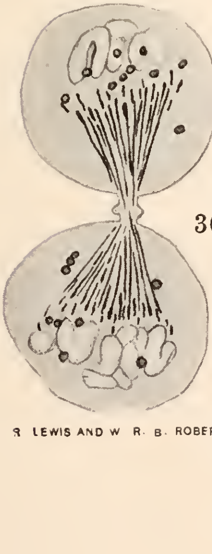
33



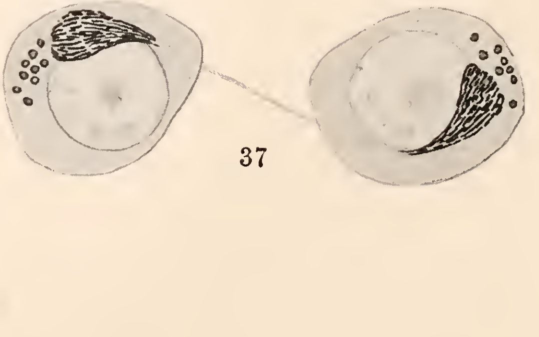
34



35



36



37

M. R. LEWIS AND W. R. B. ROBERTSON.

PLATE V.

FIG. 38. J. G. Young spermatid, centrosome divided. Nebenkern granules. Leitz Binoc. 4b × 1/12 lens.

FIGS. 39, 40. Unstained. Young spermatid. Neutral red granules. Thread-like appearance in the nebenkern. Zeiss 6 oc. 2 mm. lens.

FIGS. 41, 42. J. G. Nebenkern dividing is now granular. Neutral red granules. Leitz 4b × 1/12 lens.

FIG. 43. Unstained. Nebenkern divided. Zeiss 6 oc. 2 mm. lens.

FIG. 44. Unstained. Nebenkern sacs with the centrosome close to the nucleus. Neutral red granules. Zeiss 6 oc. 2 mm. lens.

FIG. 45. Unstained nebenkern sacs are slightly elongated. Zeiss 6 oc. 2 mm. lens.

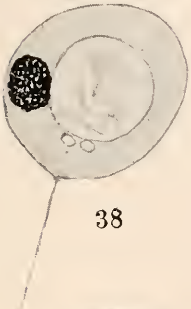
FIG. 46. J. G. Nebenkern sacs elongated. Axial filament. Double middle piece body. Neutral red granules. Zeiss 6 oc. 2 mm. lens.

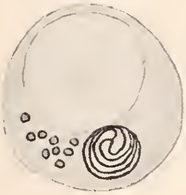
FIGS. 47, 48. J. G. The tail failed to develop but the mitochondria elongated and formed the double thread as usual, although the double thread is wound round and round within the cell. Leitz Binoc. 4b×1/12 lens.

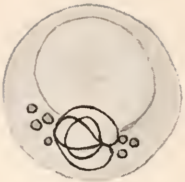
FIG. 49. N. R. The cytoplasm of the tail is gathered into nodes and the neutral red granules appear. The mitochondria is now in the form of two homogenous long threads. Zeiss 6 oc. 2 mm. lens.

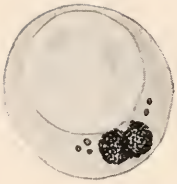
FIGS. 50, 51. J. G. Later stages of the spermatozoön.

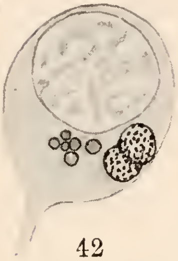
PLATE V.

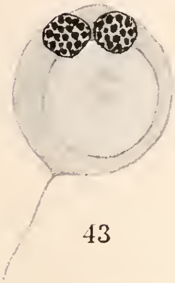

38

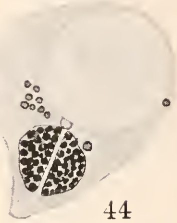

39

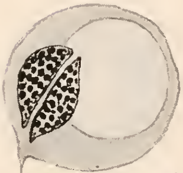

40

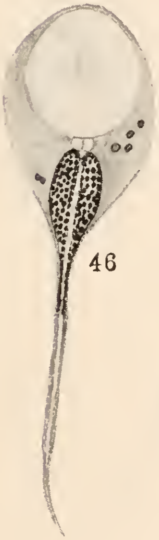

41

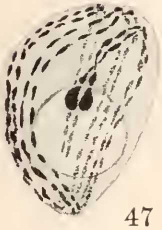

42

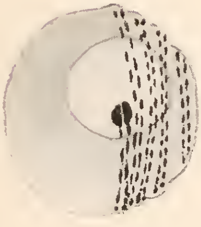

43

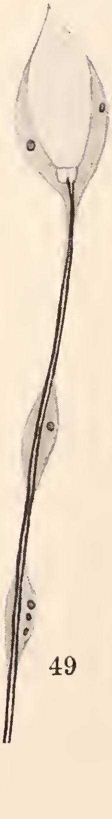

44

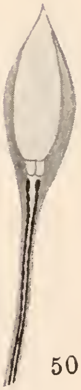

45

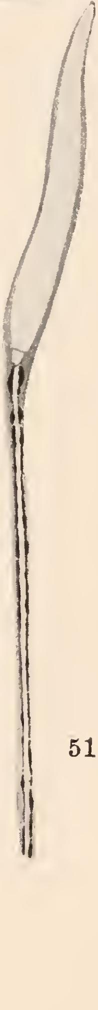

46


47


48


49


50


51

M. R. LEWIS AND W. R. B. ROBERTSON.

NOTES ON THE PHYSIOLOGY OF FUCUS SPERMATOZOIDS.

W. J. ROBBINS

Cornell University

The notes here presented are of work conducted at the Marine Biological Laboratory, Woods Hole, Mass., during the summers of 1912 and 1913 at the suggestion of Dr. B. M. Duggar, to whose kindness I also owe the opportunity and pleasure of working there.

Strasburger (1) states that spermatozoids of *Fucus*, passing at a distance of one or even two diameters from the egg, turn from their path and rush toward the egg. This attraction, he says, is a chemical one and is due to some substance secreted by the egg which conditions the direction of motion of the spermatozoids. Strasburger also states that healthy spermatozoids are strongly negatively phototactic. These two phenomena of phototaxy and chemotaxy are used by Strasburger as a basis upon which to account for the meeting of the spermatozoids and the ova as follows:

The ova have a density greater than water and sink. The spermatozoids, as they are negatively phototactic, swim downward bringing them into the region where the chemotactic influence of the eggs is sufficient to complete the union of the spermatozoids with the ova. Strasburger made no attempt to discover the chemotactic agent.

Bordet (2) also attempted an explanation of the same problem. He filled a capillary tube with crushed ova, immersed the tube in a drop of sea water containing *Fucus* spermatozoids, and watched for evidences of attraction, but found none. From this he concludes that the ova exert no chemotactic influence on the spermatozoids. He observed, however, that the spermatozoids were very sensitive to contact, clinging with one cilium to the glass slide or any solid object as, for example, a capillary tube. Bordet also states that *Fucus* spermatozoids are not phototactic, being neither attracted nor repelled by light. He believes the meeting of the eggs and spermatozoids is due to chance.

In brief, Strasburger states that *Fucus* spermatozoids are negatively phototactic and are chemotactic to the ova. Bordet asserts that they are not phototactic and that there is no chemotaxy.

Material and Methods.

The diœcious form, *Fucus vesiculosus*, was used in the experiments.

To secure spermatozoids or ova for the investigation the methods described by Strasburger (1) were followed.

Inasmuch as fresh material could not be secured daily, some method of keeping the *Fucus* in good condition was desired. Sinking the material in the sea was found to yield active spermatozoids for only a day or two after bringing it in from its habitat. *Fucus* plants wrapped in towels wetted with sea water and kept in an ice chest yield active spermatozoids for at least five days after being brought in. The antheridia from fruiting tips kept in sea water in the ice chest for the same length of time were exuded on drying, but the spermatozoids were inactive and lay inert in the antheridia. This difference between the two treatments is probably due to differences in oxygen supply. Strasburger advises shipping fruiting *Fucus*, desired for active spermatozoids and living oögonia, in large amounts of sea water, but it appears that wrapping the *Fucus* in wet cloths and icing it would be preferable.

Experiment on Chemotaxy.

In investigating chemotaxy Pfeffer's capillary tube method was used. The capillary tubes mounted in a drop of sea water containing the spermatozoids were examined under the microscope for about three quarters of an hour for change in direction of motion of the individuals as they passed by the mouth of the capillary tube and for collection of the spermatozoids in the tube as described by Pfeffer and others for various Pteridophytes. The majority of the substances, on account of the strong negative phototaxy of the spermatozoids, were also tested by placing the mounts in the dark and examining them after ten minutes, and again in from twenty-five to forty minutes, for collection in or about the tube.

The following substances made up in sea water were used in the concentrations noted:

Substance.	Concentrations.				
Lactic acid	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Malic acid	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Butyric acid	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Citric acid	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Potassium oxalate ($K_2C_2O_4$)	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Ethyl butyrate			0.08%,	0.008%	
Urea	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Sodium potassium tartrate	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Ethyl alcohol	95%,	9.5%,	0.95%,	0.095%	
Cane sugar	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Peptone	2%,	0.2%,	0.02%,	0.002%	
Potassium hydroxide	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*,	0.0001 *Mol*
Hydrochloric acid	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*,	0.0001 *Mol*
Potassium nitrate	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Diabasic potassium phosphate	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Potassium iodide		0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	
Ammonium sulphate		0.6 *Mol*,	0.06 *Mol*,	0.006 *Mol*,	0.0006 *Mol*
Hydrogen peroxide	3%,	0.3%,	0.03%		
Sodium sulphate	1.0 *Mol*,	0.1 *Mol*,	0.01 *Mol*,	0.001 *Mol*	

No reactions which could be attributed to chemotaxy were observed with any of the above substances except the acids.

It was found that with a molecular solution of the organic acids in the capillary tube the spermatozoids were killed in the course of a few minutes. Where 0.1 *Mol* acid was used there was in some cases a collection of spermatozoids around the mouth of the tube apparently attached to the cover glass or slide by one cilium and these vibrated slowly. This collection in a few cases was visible to the naked eye after an hour as a yellow halo about the mouth of the tube. With 0.01 *Mol* solution in some of the trials there was a collection of the spermatozoids in the tube after 45 minutes or more; 0.001 *Mol* had no effect. The accumulation around the mouth of the tube containing 0.1 *Mol* acid and the collection in the tube containing 0.01 *Mol* acid only occurred when sea water was used in which the spermatozoids were very active and numerous.

Such results might be interpreted as cases of chemotaxy.

Kusano (3) has described similar reactions of the swarmspores of Myxomycetes to acids. He attributes them to chemotaxy. An examination of individual spermatozoids, however, showed no change in the direction of their movements passing the mouth of the tube, the effects could by no means always be obtained, and much the same result was observed in one case with HCl. I believe that the results could be interpreted as a toxic phenomenon as explained below.

In the case of the molecular concentration, the diffusing acid is sufficiently strong to kill or paralyze the spermatozoids throughout the whole mount. With acid of 0.1 *Mol* concentration the diffusing zone of the acid which is toxic is not so wide as in the case of acid of the molecular concentration and only those spermatozoids are rendered non-motile which pass comparatively close to the mouth of the tube; hence the collection observed after a time around the mouth of the tube. At the concentration of 0.01 *Mol* the diffusing acid is not toxic, and only those spermatozoids are rendered non-motile which pass comparatively close to the mouth of the tube; hence the collection observed after a time around the mouth of the tube. At the concentration of 0.01 *Mol* the diffusing acid is not toxic, and only those spermatozoids which enter the tube, as some must from several hundred of individuals in a mount, are paralyzed and remain there. Acid of 0.001 *Mol* concentration is below the toxic concentration and we have no effect. We do not find the effects noted above in every mount where the acids are used because there may be an accumulation of all active spermatozoids due to negative phototaxy on the side away from the source of light, in which case they do not meet the acid diffusing from the tube; or there may be too few individuals in the mount to show distinct collection; or they may not be actively motile due to the high temperature or old material.

If the results noted are due to a toxic effect of the acids on the spermatozoids, it would be expected that other substances, such as potassium hydroxide and ethyl alcohol should also produce the reaction. While no response to those substances was noticed, this may have been due to poor material or to too high a temperature at the time of the experiment. In either case the spermatozoids would be less active and no accumulation would occur. At the time the work was done the relation between toxicity and chemotaxy was not considered and further work on this phase was not attempted. It would appear that the subject should be investigated.

Experiments on Phototaxy.

That *Fucus* spermatozoids are negatively phototactic is very evident. Under the microscope the large majority will be observed to swim away from the light and in a short time practically all the active spermatozoids will be found on the side away from the light. If a capillary tube lies parallel to the window the spermatozoids in the drop will collect against the tube on the window side, blocked in their movement away from the light. If mounted on a slide containing a bright spot of light in a dark field, there is no collection in the bright area as Englemann (4) found for *Euglena* under the same conditions. The spermatozoids pass in and out of the bright area with no apparent response.

A slide holding a drop of sea water one fourth inch in diameter was placed about two feet from a north window. In less than five minutes practically all the active spermatozoids were found crowded on that side of the drop away from the window. It seemed possible this might be due to gravity.

Two slides were arranged as above, one inclined toward the window and one tilted away from it. In both cases the spermatozoids were found crowded on the side away from the window. In both cases the spermatozoids swam away from the light, but in one case they swam with gravity and in the other against gravity. A liter beaker half filled with sea water yellow with spematozoids was set on a shelf ten feet from a north window. In ten minutes far more active spermatozoids were found in a drop taken from the side away from the window than were found on the lighted side.

Influence of Temperature on Activity.

From indications during the experiments on chemotaxy it was observed that temperature had a very considerable effect on the activity of the *Fucus* sperm and that the optimum temperature is probably relatively low. The experiment below is suggestive.

Sea water yellow with sperm was placed in two vials. One vial was placed in an ice-water bath at approximately 4° C. and the other was left at room temperature of 25°–27° C. Portions were removed from time to time and examined for activity.

Begun at 11:00 A.M.

Hours.	Time.	4° C.	25°–27° C.
½	11:30	More active than at 27°.	Active.
1	12:00	Many active.	Few active.
1½	12:30	Many active.	Few active.
2¼	1:15	Many active.	One or more in microscope field moving slowly.
3	2:00	Many active.	One or more in microscope field moving slowly.
4	3:00	Many active.	One or more in microscope field moving slowly.

Summary.

Using the Pfeffer capillary tube method of determining chemotaxy, it was found that certain acids cause collection of *Fucus* spermatozoids. It is suggested that this may be explained as due to toxicity and not chemotaxy. *Fucus* spermatozoids are negatively phototactic. Low temperature is favorable to the activity of *Fucus* spermatozoids.

LITERATURE CITED.

1. **Strasburger, E.**
'84 Das Botanische Practicum, Erste Auflage. P. 392.
2. **Bordet, Jules.**
'94 Contribution a l'étude de l'irritabilité des spermatozoides chez les Fucacées. Bull. de l'Acad. Roy. de Belgique, 3 série **27**: 888–896.
3. **Kusano, S.**
'09 Studies on the Chemotactic and other Related Reactions of the Swarm spores of Myxomycetes. Tokyo Imp. Univ., Agr. Jour. **2**: 1–83.
4. **Engelman, T. W.**
'82 Über licht und Farben perception niederster Organismen. Arch. f. d. ges. Physiol. **29**: 396.

NOTE ON THE GALVANOTROPIC RESPONSE OF THE EARTHWORM[1]

A. R. MOORE AND F. M. KELLOGG,

Biological Laboratory of Bryn Mawr College, Bryn Mawr, Pa.

Since Blasius and Schweizer[2] first found that many animals, both aquatic vertebrates and invertebrates, orient themselves characteristically to the electric current, the galvanotropic response has been studied carefully by a number of investigators in paramecia, motile algæ, medusa strips and tentacles, certain crustacea and salamanders. In general it may be stated that under normal conditions, paramecia, motile algæ, and pieces of medusa tend to move toward the kathode, while vertebrates and crustacea which are galvanosensitive, progress toward the anode and avoid the kathode either by turning away from it or by walking backward. Loeb[3] and his collaborators account for the coördinated movements of vertebrates and crustacea under the influence of the electric current by suggesting that they are due to changes in the tension of associated muscle groups, viz: flexors and extensors.

It seemed that the earthworm, *Lumbricus terrestris*, with its simple locomotor system of circular and longitudinal muscles offered favorable material for testing this idea. The following is an account of the response of this animal to the constant electric current, based on a large number of observations made on different individuals at different seasons of the year. It is true that Blasius and Schweizer noted the galvanotropic response of *Lumbricus* but unfortunately limited their description to the stimulating effects of the "make" shock and the final crawling of the animal to the kathode, consequently omitting mention of the significant intermediary stages due alone to the flow of the constant current.

In our own experiments, an animal to be tested was put into a hard rubber trough, 5 cm. × 5 cm. × 20 cm. and containing 1 cm. depth of tap water. The current, from the ordinary lighting circuit, passed through a water rheostat, pole changer, milliammeter and key, and was received at either end of the trough by

---

[1] Drawings by Mary Cline.

[2] Blasius and Schweizer, *Pflueger's Archiv*, Bd. 53, S. 493.

[3] Loeb, J., *Pflueger's Archiv*, Bd. 66, S. 439.

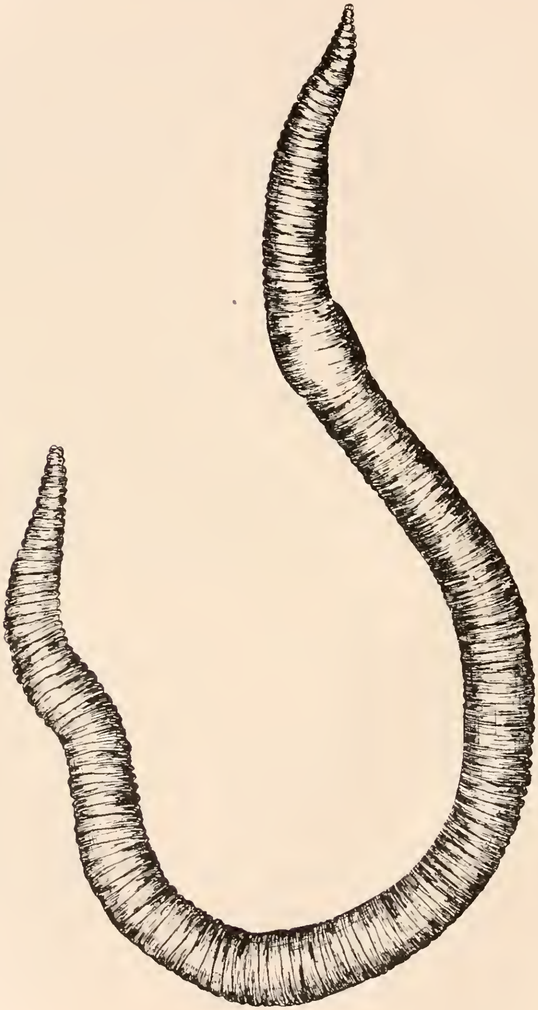


FIG. 1.

non-polarizable electrodes made of wet filter paper. The strength of current used varied between 20 and 100 milliamperes per square decimeter, voltage 110. As soon as the current was made the worm began to orient itself and in a few seconds the anterior and posterior ends were directed toward the kathode, so that the animal took the form of a U with the concave side toward the kathode (Fig. 1). In this situation it continued to make writhing and progressive movements, and therefore the figure varied somewhat from time to time. If, by reason of excessive movements, any part of the worm showed anodal curvature, the latter was instantly corrected by contraction of the muscles on the kathodal side. Because of the anterior end of the worm being always the more vigorous in its movements, the animal ultimately succeeded, as a rule, in crawling to the kathode.

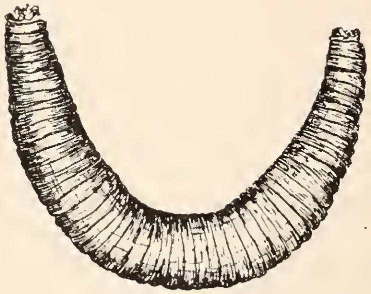


FIG. 2.

If it were cut into pieces 3 to 4 cm. long, these pieces when placed transversely in the trough and subjected to the action of the constant current, continued to orient themselves in the same fashion as an entire worm (Fig. 2) but progressive movements were absent. When the current was reversed, the specimen, either entire or sectioned, immediately responded by turning its ends towards the new kathode. The delicacy of the response was lost in fatigued animals.

Since the earthworm has but two muscle systems with which to accomplish its locomotion, viz: circular and longitudinal, it is obvious that one-sided movement or bending can be brought about only by unequal contraction of the longitudinal muscles on the two sides. It would seem that this is a case in which an entire animal responds to the electric current in the same fashion as did Bancroft's[1] medusa tentacles and bell strips. Since no locomotor limbs are involved in the earhtworm's reaction its response accords in as simple a way as possible with Loeb's theory of galvanotropism. We may therefore conclude that the constant current produces the effects described by increasing the tension of the longitudinal muscles on the kathodal side of the worm, which results in this part being more strongly contracted than the anodal region.

---

[1] Bancroft, F. W., *Journ. Exp. Zool.*, Vol. I., p 289.

PALM AND SOLE STUDIES.

HARRIS HAWTHORNE WILDER.

TABLE OF CONTENTS.

I. Introduction 135
II. A Primitive Palm Print 153
III. The Border Region of the Plantar Friction-Skin 157
IV. The Reduction of Line C 171
V. Friction-Skin Correspondence in Pygopagi 211
VI. The Heritability of Friction-Skin Characters 227
VII. Bibliography of Friction-Skin Configuration 245

I. INTRODUCTION.

In the study of the details of the configuration of the friction-ridges found covering the surfaces of human palms and soles there opens up a field of the greatest value for the biologist. Varying greatly individually, though constant throughout the life of a given individual; still following the lines laid down for them in more primitive mammals, yet modified and varied as the result of mechanical causes; showing markedly and with certainty a direct inheritance from the immediate parents as well as from generations more remote; they may be used with profit by the morphologist, the ethnologist, or the student of genetics, while, as the surest and most positive characters of an individual, they may serve the authorities in the identification of a human body, living or dead.

A great advantage in the study of these parts lies in the ease with which a print of the surfaces may be taken, thus furnishing a permanent record, accurate in even the minuter details, and easily filed away. These prints, with the ridges marked in black upon a white background, and reduced to a perfectly plane surface, are much easier to study than are the real objects; laid out upon a large table they may be compared with ease; they are always ready for reproduction where desirable. Undeniably the patterns are complicated, and many new conceptions, and the new terminology which expresses them, confront the beginner, as in any new field; but this much once accomplished there opens up to the investigator an almost endless series of new phenomena the study of which, in the few years during which the subject has received special attention, has been no more than begun.

As this paper is intended in part as an invitation, or perhaps a propaganda, for more work in this subject, a bibliography of the entire subject is appended, with some suggestions as to the character of the separate titles. It may not come amiss, also, to give in this place a key to the method of formulation now in use, which, although somewhat artificial in the sense that it does not have much morphological significance, is a convenient way in which to express the fundamental conditions found in a given palm or sole, and as such, has already been extensively used.[1]

In the palm the starting-points of this system are four *triradii* (Galton's *deltas*) which lie at the bases of the four fingers, and may be designated as *A*, *B*, *C*, and *D*, the first situated beneath the index finger, and so on. As in all triradii, these points form each the meeting place of three radiating lines, at approximately 120° from each other, and of these three *radiants* two short ones pass obliquely upwards (distally), defining a small *digital area*, while the third follows a longer and quite variable course across the palm. These latter are the four *Main Lines*, designated by the same letters as are used for their triradii of origin, and as their position indicates the configuration of the entire palm, a simple method of describing their course is of first importance.

To "interpret" a given palm it is first necessary to locate the four digital triradii, *A*, *B*, *C*, and *D*, and then to trace from these centers their three radiants, and more especially the main lines, following them across the palm wherever they may lead, never crossing a ridge. Often, in this pursuit a single ridge may be followed almost the entire distance; again, the ridge that is being followed may come to an end, yet the direction be immediately taken up with a new one, upon which the line may be continued. Where a ridge forks, and thus allows two or more possible courses, the most distal one should be taken. If, now, these courses be

---

[1] Cf. the recent papers of Schlaginhaufen, 1906; Loth, 1906, and the two papers of Wilder on "Racial Differences," 1904, 1913; cf. also the exposition of the method in Martin's new "Lehrbuch der Anthropologie," Jena, 1914, pp. 360–367.

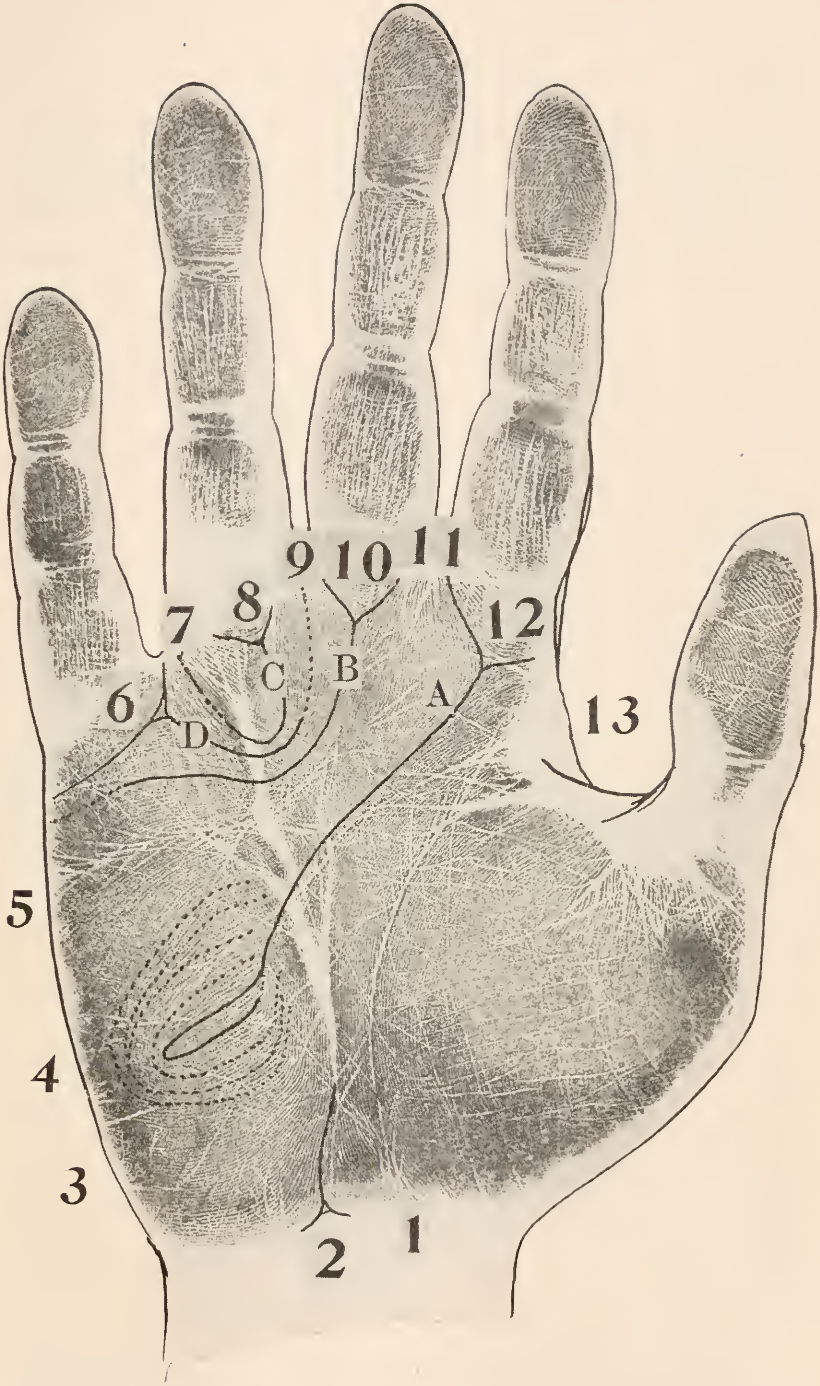


FIG. 1. Print of a left hand, showing method of "interpretation," as explained in the text. The print shows the four *main lines*, and the numbers arbitrarily assigned to the different parts of the margin. Formula: 9·7·5·4. Line *A* becomes involved in a hypothenar pattern, giving it the value of 4.

marked, while being followed, by a colored pencil, of red, or some other conspicuous color, or by ink, the palm will appear like those shown in Figs. 1 and 2, except that the result will be naturally more or less unlike either model.

As the triradii of origin never vary much in position, the general course of the main lines may be given by determining with some precision their termini, that is, the points at which they issue from the margin of the friction-skin area. This is easily accomplished by designating the several regions and points along the margin by an arbitrary system of numbers, as here shown, using the numbers 1 to 13. In this the more definite points, like triradii or pattern-cores, are designated by even numbers, and the intervals between these by odd. Thus 2 indicates the *carpal triradius*, lying on the proximal margin at the middle of the wrist. When not actually present, the location of this point is equally well determined by a parting of the lines towards the radial and ulnar sides. 4 indicates the *hypothenar* pattern, a conspicuous feature present in about 20 per cent. of white hands; when the pattern is wanting, this number is not used. The numbers 6, 8, 10, and 12 indicate the four digital triradii, but in the reverse order, beginning with triradius *D*. The odd numbers are not so precise, and designate the entire lengths of margin between the points just mentioned. 1 means any termination upon the radial side (thumb-side) of the carpal triradius; 3 begins at this latter point and runs up along the ulnar side as far as the hypothenar pattern, and 5 lies between this pattern and triradius *D* at the base of the little finger. When an hypothenar pattern is not indicated the distinction between 3 and 5 is somewhat uncertain, but in general, if the entire outer margin of the palm between the lower outer corner (proximal ulnar) and triradius *D* be divided into thirds, the lower, or proximal third is 3, while the distal two thirds are 5. The boundary between these two numbers thus corresponds to the point of location of 4 (hypothenar pattern) when present. The numbers 7, 9, 11 and 13 designate the spaces between the fingers, 7 being that between the little- and ring-fingers, and so on.

Thus, given these arbitrary values for the parts of the margin, it will be seen that in Fig. 1, line *D* crosses the margin at 9, *C* at 7, and *B* very high up along 5. Line *A* becomes involved in the hypothenar pattern, indicated by the digit 4. The entire formula is thus, in the natural order, $4 \cdot 5 \cdot 7 \cdot 9$. In Fig. 2 lines *B* and *C* become confluent, so that the termination of each is designated by the number indicating the origin of the other, and the formula

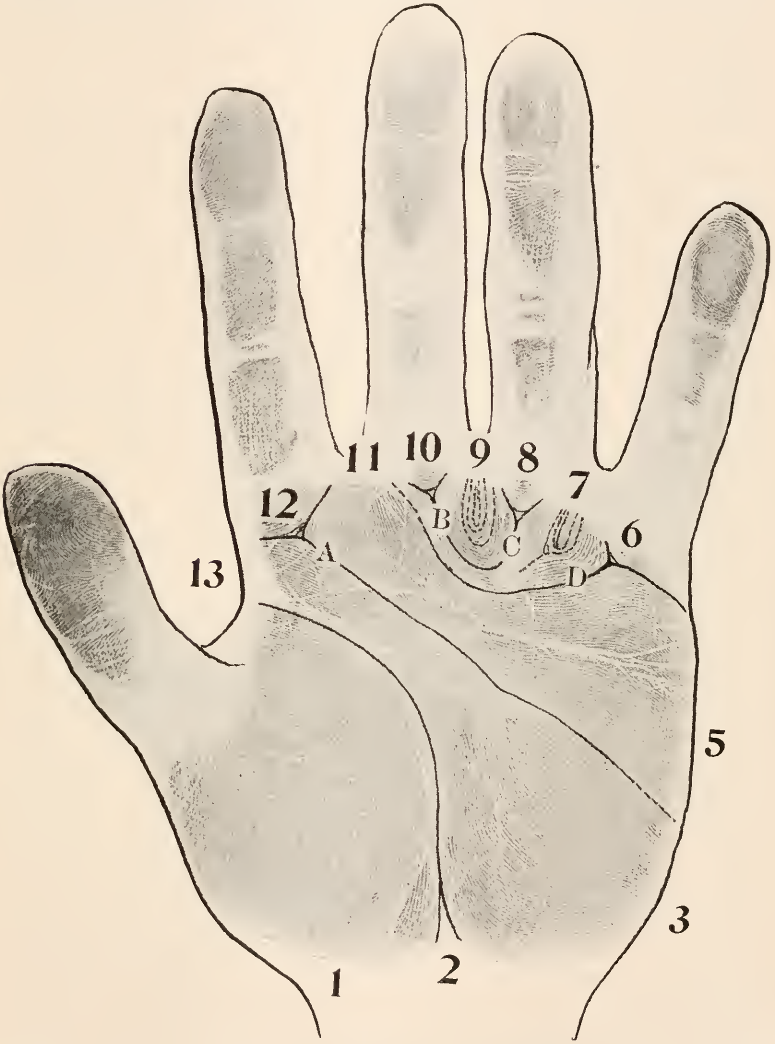


Fig. 2. Print of a right hand, marked as in Fig. 1. Formula: $11 \cdot 10 \cdot 8 \cdot 5$. Here the main lines *B* and *C* are confluent, and may each be considered to end in the triradius of origin of the other; that is, line *B* ends in triradius *8*, and line *C* in triradius *10*. The third and fourth interdigital patterns are present, the latter with a triradius belonging to it. These features may be added to the formula in giving a complete description.

will read 5·8·10·11. In this hand, also, is shown a case of divergent ridges along the course of line *A*, a common phenomenon, so that by following different ridges the line might be made to terminate almost anywhere along a considerable extent of margin. Following the rule above stated, however, that of carrying a line as far distally (towards the finger-tips) as possible, the line is made to terminate at 5.

In this way any human palm may be formulated by the use of four numbers, which indicate the conditions so nearly that one cannot be far out of the way if he should try and draw a given palm from the formula alone. This can be easily proven by anyone who knows the system and makes the attempt, using the formula only, and withholding the original print until the drawing is completed. This formula makes no attempt to indicate any features other than the course of the four main lines, yet, in point of fact, the presence of a pattern is sometimes indicated, as in the case of Fig. 2, where the fusion of lines *B* and *C* encloses a small area between the middle- and ring-fingers, and makes a looped pattern there a necessity.

When a large number of formulæ are accumulated they may be readily arranged in numerical order, subdividing first by the first number, and so on. For practical purposes, however, it is found better to reverse the entire formula, beginning with the number representing the course of line *D*, since with this latter there is not only more precision in termination than in the case of line *A*, but also line *D* is more variable, and thus furnishes more classes for the first subdivision. Thus the formulæ for the two cases here illustrated should read respectively, 4·5·7·9 and 11·10·8·5, the first coming before the second in a numerical list.

Corresponding to the results of a morphological study of the *patterns*, which shows them to have first developed on the raised surfaces of the eleven typical pads (Whipple, 1904), traces of this number are to be expected in their proper places. The five apical patterns appear on the balls of the five digits, the center or core of each coinciding with the rounded apex of the raised area. In their most primitive form they appear as concentric circles or ovals, or some modification of this form, limited by two triradii (deltas), the "*whorls*" of the finger-print system; the reduction of either one of the original triradii transforms the whorl into a *loop*, ulnar or radial in accordance with the triradius that is suppressed; and this figure in turn becomes an *arch* by the suppression of the remaining triradius. The low arch, without appreciable core, is thus the final stage in the reduction process of a finger pattern. The fact that all possible stages along several lines of degeneration are found in man indicates the reduction in functional value as friction organs of these once so vital parts.

The six patterns of the palm proper consist of the four *interdigitals*, lying just proximal to the intervals between the fingers; the *thenar*, and the *hypothenar*. Of the four interdigitals the first (at the radial end of the series) lies below the wide interval between thumb and index finger, and is found usually in close connection with the thenar, the two forming loops opening in opposite directions, and with two triradii between them. This pattern-complex is rare in the white race; but much more frequent in some others (*e. g.*, the Maya-Quichés of Yucatan; Wilder, 1904). The three remaining interdigitals appear below the finger intervals, the second lying between triradii *A* and *B*, the next (third) between *B* and *C*, and the fourth between *C* and *D*. The one between triradii *A* and *B* (second) is the rarest of the three and the fourth, between *C* and *D*, is the commonest. Furthermore, this last is frequently provided with a triradius of its own, aside from *C* and *D*, a peculiarity occasionally, but not so frequently, met with in the other cases. The third pattern, between *B* and *C*, is liable to be confused with a "false pattern" caused by certain configurations of the ridges of the palm, but easily distinguished from the real one by its position in a depression rather than on an elevation (Whipple, 1904). The three mounds of this region are easily seen in most hands by bending the fingers back and looking across the palm in various directions. They are the plainest in children and in the fetus they are often extremely conspicuous (Retzius, 1904).

As might be expected, a human hand presenting all eleven patterns is quite rare, yet does occur. Such a case, found in the right hands in twin boys, has been already figured and described by the writer.[1]

As each of the four interdigital pads (or patterns) possesses typically three triradii of its own, with four for the third, it is

---

[1] Wilder, *Anat. Anz.*, Bd. XXXII., 1908, pp. 194, 195.

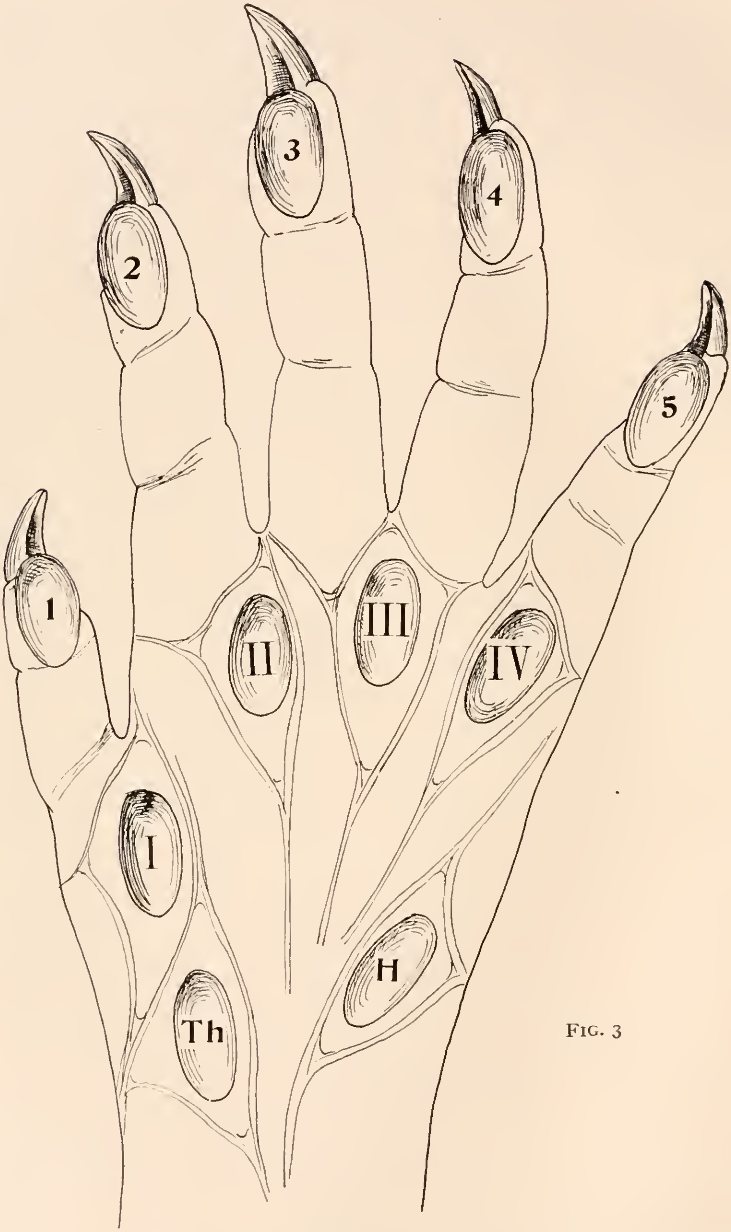


Fig. 3

Figs. 3–5. Diagrams giving the morphological explanation of the friction-ridge configuration as found in the Primates, and especially in man. Fig. 3 represents the condition in primitive walking mammals, and follows closely the condition

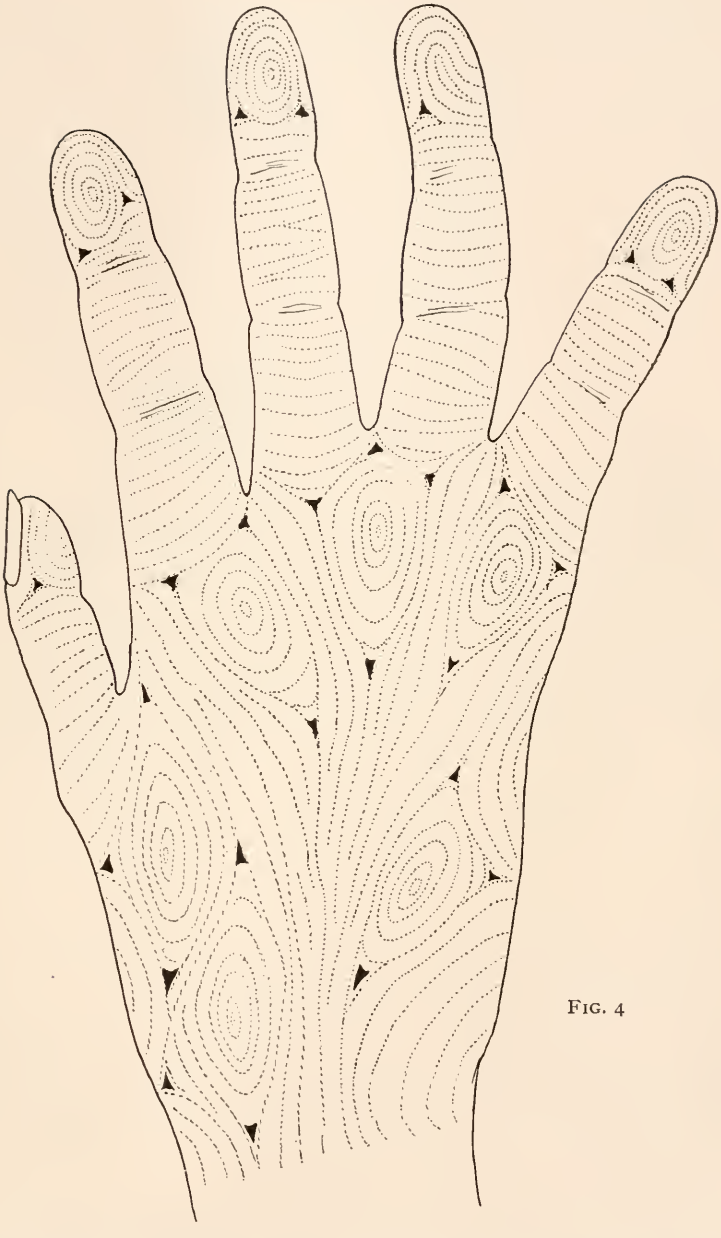


FIG. 4

found in *Microtus*, a field mouse and *Crocidura*, a shrew. The contact with the ground comes upon eleven walking pads; *five* apical or terminal, 1–5, *four* interdigital, I.–IV., a thenar, Th, and a hypothenar, H; *eleven* in all. These are surrounded by folds of skin, two for the apical pads, four for the third interdigital, and three

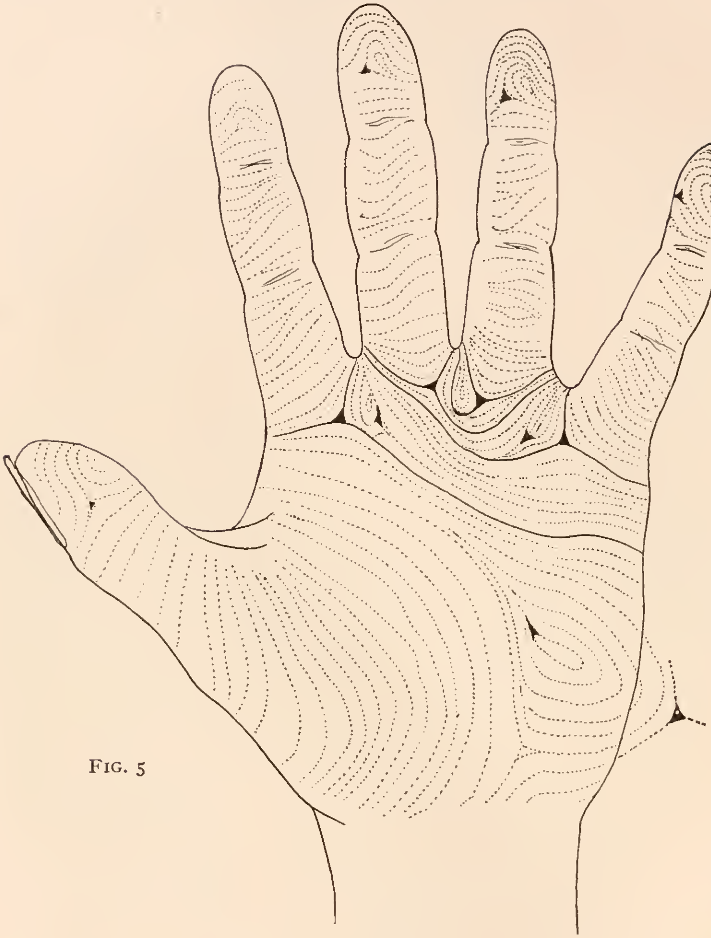


FIG. 5

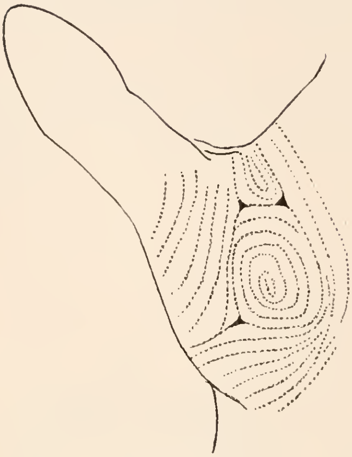
for each of the others, and where these folds come together there are formed *triradii*, or centers from which folds radiate in three directions.

Upon this original surface certain epidermic units (scales?) which are undoubtedly very primitive, and were there before the formation of pads and folds, become definitely arranged in concentric circles over the pads, and in lines along the folds. Nearly every stage in this rearrangement may be found by looking over the more primitive mammals, especially marsupials and lemurs. The separate rows of units tend to unite to form ridges, which possess an important function in increasing the friction, and thus preventing slipping.

In monkeys, Fig. 4, the pads and folds are flattened down to an approximately level surface, but the arrangement of the ridges still indicates the former condition. The *relief* has become a *picture*.

In man, Fig. 5, the reduction in functional importance of these friction-ridges allows all forms of degeneration, both in the individual patterns and in the ridges

Fig. 5*a*.



as a whole. There is also shown a marked tendency for all the ridges to shift from the longitudinal position of the apes to one more nearly transverse, and this tendency is much more marked in right than in left hands, evidently corresponding to use, and recording the change from the grasping of tree boughs to the holding of tools and other objects.

While all degrees of the loss of the original configuration and the assumption of transverse ridges may be found in different human hands, it is seldom that so many of the mound patterns are retained as in Fig. 5 (Coll. No. 90). The formula, however, 11·9·7·5, indicated the establishment of the transverse position in the interdigital region. Fig. 5*a* shows a very primitive Thenar region in a Liberian negro. (Coll. No. 571, taken by F. Starr.)

evident that in the human species a very considerable reduction of these points has taken place. There are left in all cases, however, the four main triradii, which are so constant as to allow their use as the starting points in palm formulation although it is by no means certain that they are in all cases strictly homologous. Thus, from Figs. 3 and 4 it is seen that the second interdigital pattern has originally two distal triradii, either of which might persist as triradius *A*, and it is likely that the one that appears is sometimes one and sometimes the other of the original two. Other of the missing triradii appear occasionally somewhat lower down on the palm (proximal to the pattern) especially in connection with the fourth pattern.

A definite hypothenar pattern appears on about 20 per cent. of hands of the white race, but the occurrence differs considerably racially and in some may be either more or less frequent. It is occasionally found in its more primitive form, as a whorl with the three original triradii, but it displays a tendency to become drawn out along one axis and form an extensive spiral or S-formed figure.

As is well known, the exact and detailed configuration of the five apical patterns, the "finger-prints" of identification bureaus, was employed by the late Sir Francis Galton (1892, 1893, 1895, etc.) as the basis for his system of personal identification, and, within the last twenty years, has become scientifically developed by certain police investigators, notably Sir Edward Richard Henry. At the present time the "finger-print system" has been introduced into all civilized countries, and its scope is steadily increasing. As the friction-skin configuration offers an abso-

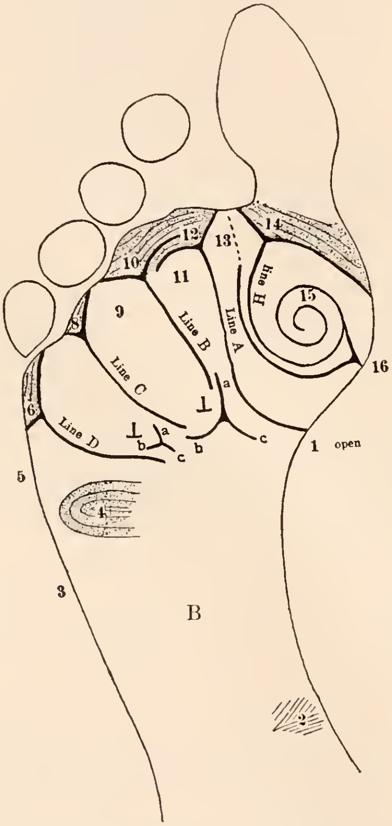


FIG. 6. Diagram of a human sole, with the margins numbered to correspond to the palms in Figs. 1 and 2, and marked with the *main lines*. From an article by the author in *Pop. Sci. Monthly* for September, 1903. By permission.

lutely sure test, and the only one, for the identification of a human body, alive or dead, it is certain that this use of the apical patterns will be followed by a similar employment of the entire palmar and plantar surfaces, as advocated by the present author since 1902, and at this writing it is a pleasure to note that sole-prints have recently been put in use in a Chicago maternity hospital for the identification of the babies.

Something on the frequency of occurrence of the six patterns upon the palm has been done in connection with racial differences in friction-skin configuration[1] and their progressive degeneracy from the simian type has been followed, in some cases along several lines, by Miss Whipple (1904, pp. 341–350).

The friction-skin configuration of the sole shows a much greater range of variability than is seen in the palm, and correspondingly its formulation presents a more serious problem. When the print indicates the four main triradii it is possible, of course, to draw the same four main lines, *A*, *B*, *C*, and *D;* using the same arbitrary numbers to designate marginal features (Fig. 6), yet this method is unpractical from a number of causes. In the first place the four essential triradii lie up in the hollow between the ball and the toes, and quite often come beyond the usual contact area, and thus are not seen in a print. Again, they are by no means as constant as in the hand, being occasionally replaced by other triradii which have survived instead of these; and still again, when the main lines are traceable, they quite often assume near their free ends a parallel course and terminate close together along the margin designated by the digit 1, thus giving little or no variety to the formula (1 · 1 · 1 · 1).

It has thus seemed more expedient to employ a distinctly different scheme for sole formulation, as illustrated in Figs. 7 and 8. The print is interpreted, that is, is marked off into areas, by the use of the main lines as in the palm, but as the lines themselves are not used otherwise than as boundaries, the tracing of their course is not so critical. Where all four main triradii are present as in Fig. 7, this interpreting is a simple matter; yet there are numerous cases in which one or more of the triradii lie above (distal to) the contact area, as in the case of triradius *C* of Fig. 8. Here there is, however, some divergence of the ridges at the upper margin, sufficient to indicate the position of the line approximately, and the case is assisted by the presence of a "lower" or proximal triradius, one radient of which practically coincides with the supposititious *C* line. Thus between the two a boundary is fixed between the areas of the fourth and the third interdigital patterns, while the second, and the boundary between it and the third are definitely indicated by means of the main triradii.

---

[1] Wilder, *Amer. Anthrop.*, 1904, 1913.

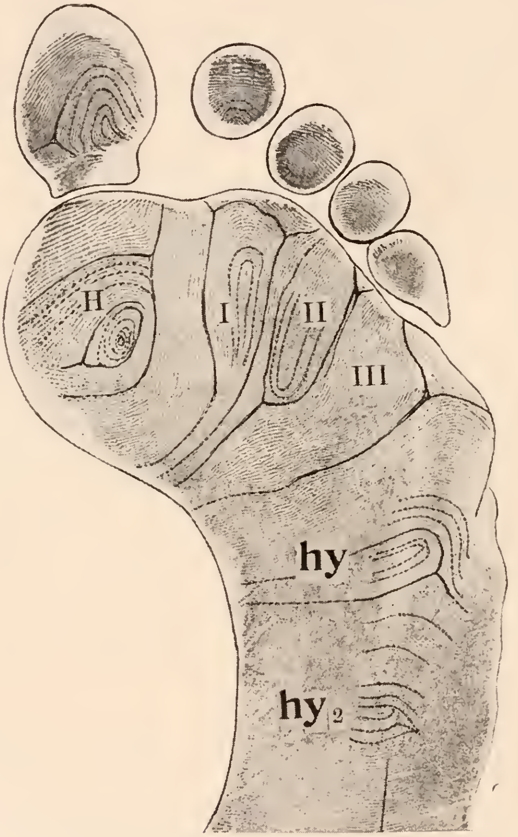


Fig. 7. Print of a right sole, showing the method of dividing the "ball" into its four interdigital areas, each of which may be separately described by formula. This method is fundamentally different from that used for the palm, as shown in Figs. 1 and 2. Formula: $W \cdot L \cdot Cl \cdot O \cdot H \cdot H2$.

Now in all cases, whether the limits are precise, as in Fig. 7, or approximate, as in Fig. 8, the "ball" of the foot is easily divided up into areas corresponding to the four interdigital patterns. Of these the first, beneath the great toe, or rather beneath the interval between this digit and the next, is the largest, and is usually covered with a definite arrangement of ridges, the hallucal pattern. It will be noted that this, the most important of the four in the foot, is the same one that, in the hand, appears only as an adjunct of the rare thenar pattern, and is thus of little importance. This difference is plainly the result of the difference in function and use of foot and hand, as in the former the main work is performed by the heel, and by the four interdigital areas placed in a row close together, while of this row the inner end, below the great toe, is used the most.

The descriptive formulation of the sole configuration is thus seen to be most advantageously accomplished by using the *areas* rather than the *lines*, and describing the condition of the patterns found upon them. To begin with, the hallucal pattern, which is used for the classification of soles into primary groups, is found in its most primitive form as the *whorl*, equipped with its three typical triradii, *A*, *B*, and *C* (Fig. 9). Either triradius may be wanting, thus allowing the ridges to open, or gush out, to use a

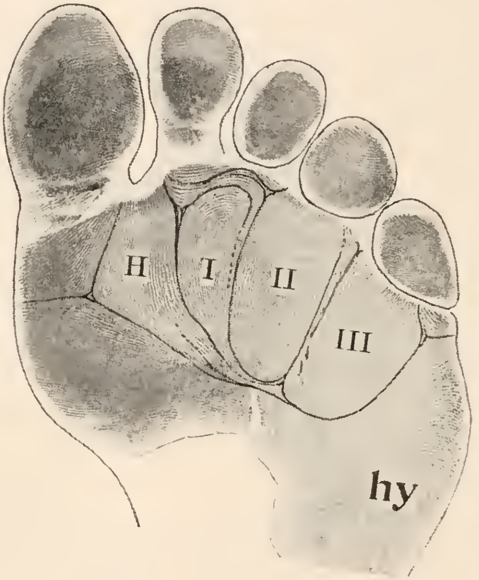


FIG. 8. Print of a right sole, showing a second case of the method of interpretation used in the preceding figure. This print exhibits the unusual case of the effacement of a definite hallucal pattern, similar to the sixth type shown in Fig. 9. Formula: $BC \cdot L \cdot Cl \cdot Cl \cdot x$.

figure which has no morphological significance, in the direction of *A*, or *B*, or *C*. The whorl, in formula writing, may be designated as *W*, and the three derived types just given, may be distinguished by the triradius that is wanting, as *A*, *B*, or *C*, respectively. Aside from these, there are compound types, where two of the triradii fail, and where the ridges flow out in two directions. Of

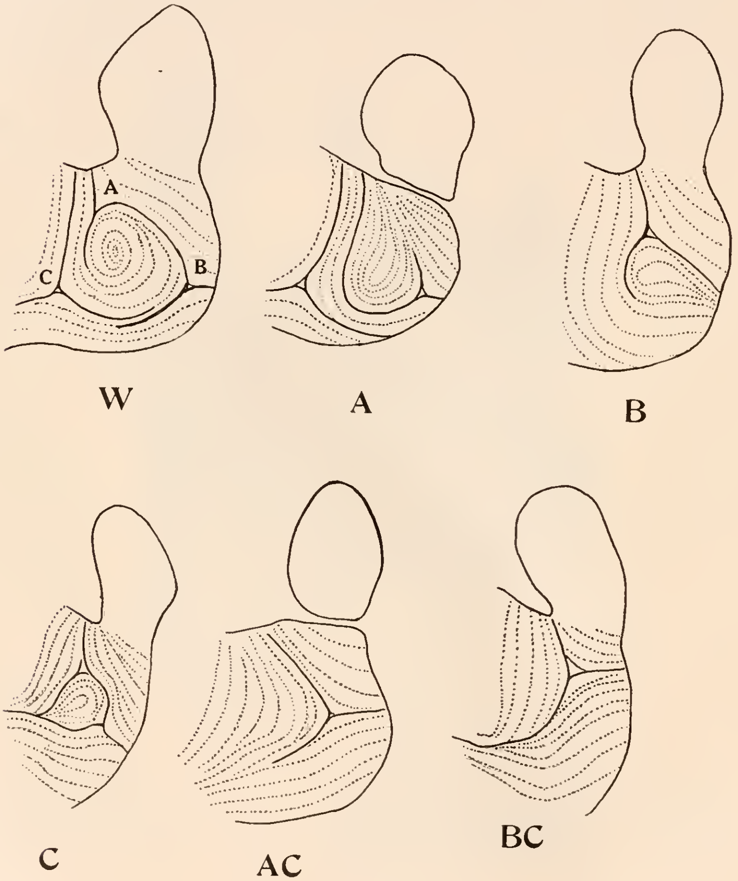


FIG. 9. Diagram showing the types of hallucal pattern. *W* is a whorl, with the three triradii all present, *A*, *B*, and *C*. In type *A* triradius *A*, the distal one. is lost, and the ridges flow out between the first and second digits. In type *B* the triradius of the name, the tibial one, is lost while *A* and *C* remain, and in type *C*, the fibular triradius (*C*) is lost, or nearly so, giving an outlet for the ridges in that direction. Types *AC* and *BC* may be interpreted as compound types, explicable from the others, especially *C*, where the two triradii *A* and *C* lie near together. In *AC* the remaining triradius is assumed to be *B*, and in *BC* it is *A*. In *AC* the remnant of the pattern lies to the left of the triradius; in *BC* below it.

these the possible types are *AC*, *BC*, and *AB*, the distinctions between which are obvious. The type shown in Fig. 7 is virtually a whorl (*W*), although it is not typical; while the type of Fig. 8 is extremely rare, and is most probably interpreted as an *AB*, with some possibility that it is rather a *BC*. The three remaining areas are conveniently designated as I., II., and III., respectively, and are characterized as either *open* (*O*), or *closed* (*Cl*) in accordance with the condition of their proximal ends. Thus, in Fig. 7, I. and III. are open, that is, they reach the inner margin, while II. is clearly closed; in Fig. 8 all three are closed, but I. is open for some distance, curves around the base of II., and abuts finally against the side of III. Occasionally, too, an area may be closed at the top, also, as area I. in both figures given, and sometimes the ridges of two areas flow into each other, that is, they are confluent. A confluence is easily marked by the designations of the areas involved, united by a + sign, as, I.+III.; I.+II.; and a closure of the distal end may be designated by an *l* (loop).

This method of formulation of the sole configuration was first attempted in 1904[1] in making comparisons of the sole prints of different human races. In this article, in addition to the formulæ, as just described, certain exponent letters with an arbitrary significance were employed to qualify the more general terms. The presence of a hypothenar pattern (usually represented by a single loop) upon the outer edge of the foot proximal to the interdigital row, was also indicated by a capital *H*, added to the rest.

Representative sole formulæ, taken from this article, are here given. The main symbols will be readily understood from the foregoing; the meaning of the descriptive exponents, which are of less value, may be found by referring to the article, pp. 253–254.

W	*O*	*O*	*O*	
A	*OCl*	*Cl*	*O*	
A.	*O*	+ 3*Cl*	+ 2*O*	*H*
Wsp	+ 3	*Cl*	+ 1	
B	+ 3	*Cl* + 3	+ 1 + 2	

In the *first* of these the hallucal pattern is a whorl, while the three succeeding areas are open fields, without patterns. The

---

[1] *Amer. Anthropol.*

second has an "*A*-loop," the commonest form of hallucal pattern, where the pattern is closed, except at the interval between the great toe and the next, where the ridges run up and attain the margin; the second area, II, has a proximal loop, probably with triradius, which confines a part of the ridges. In the *third* formula some of the ridges run in the form of a U between areas 2 and 3, and in the *fourth* a similar union involves all the ridges of areas 1 and 3. The *fifth* notes an unusual form of hallucal pattern, together with a strange mix-up in the union of areas, which will become plain from the explanation of the rest.

This method of formulating sole configuration is by no means an ideal one, but it serves fairly well the general purpose of picturing the configuration of a given sole in a few terms. A fundamentally different method has been introduced by Schlaginhaufen (1905), based upon the identity of the various triradii occurring on the sole. Each one of these is designated by an abbreviation, like "*t*9" or "*t*13," and the description is based upon the position and relations of these. This method is thus a method of description rather than a formulation, and although in many ways convenient, it depends upon absolutely exact homologies, which, in our present state of knowledge, is not possible. It thus seems better to rely upon some artificial method, which is capable of picturing the essential details of a given sole, rather than to attempt a series of homologies based upon our present incomplete knowledge.

The two proximal patterns, thenar and hypothenar, have naturally suffered much displacement from the peculiar lengthening of the proximal part of the foot to form the human heel, and a discussion of their position and homologies will be found farther on in this article. Here it may be said that the transversely placed loop, sometimes seen on the outer edge of the sole, just proximal to the row of interdigital patterns across the ball, may safely be considered the hypothenar pattern, or more probably a portion of it (Fig. 7). The thenar, rare and more or less rudimentary, as in the hand, is indicated by traces of irregularity in the lines, or an occasional loop and triradius, found upon a slightly raised area upon the inner edge of the sole, not much distal to the heel (Figs. 21 and 23 below).

II. A PRIMITIVE PALM PRINT.

Among the prints collected from college students the past year occurred an isolated case, unique in the history of human palms thus far known (Fig. 10).

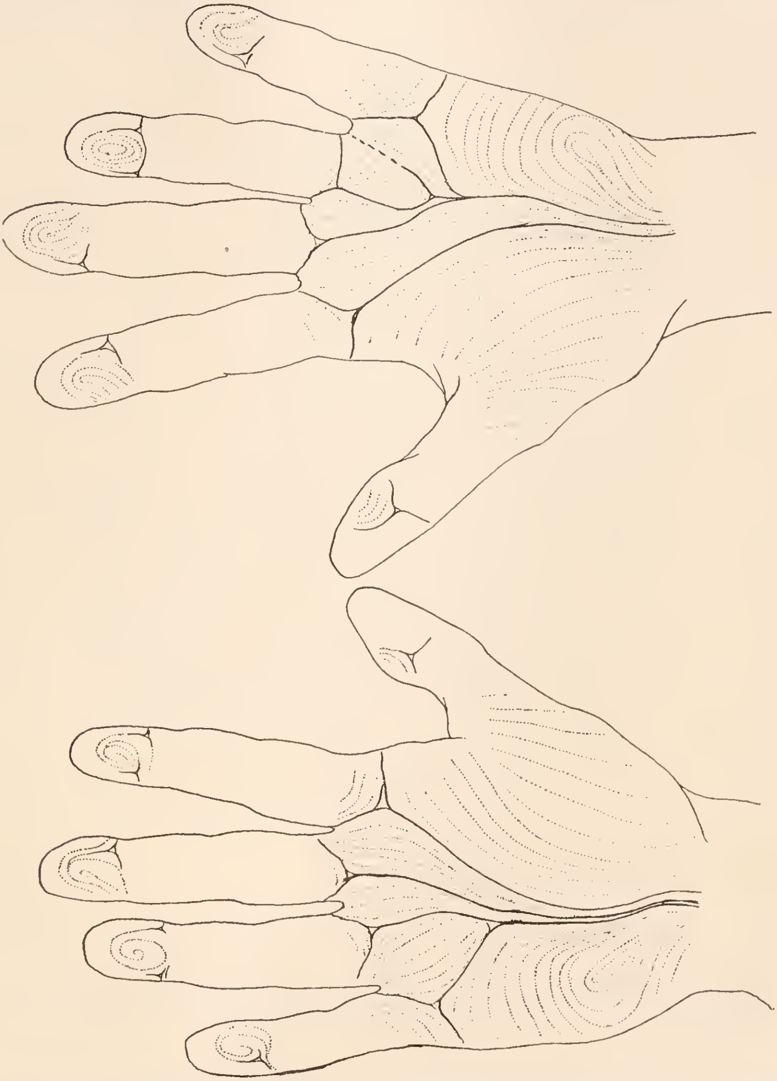


FIG. 10. Tracings taken directly from prints of the two hands of Miss ——— (Coll. No. 647), showing the simian type to a degree not even approached by any other human individual of the many hundreds thus far placed under observation by the various investigators. Formula: 1·7·1·1 or 1·1·1·1.

In the left hand of this individual, the one first noticed, all four main lines, *A*, *B*, *C*, and *D*, which proceed from the triradii at the bases of the four fingers, instead of running transversely or obliquely across the palm, as in all cases hitherto observed,

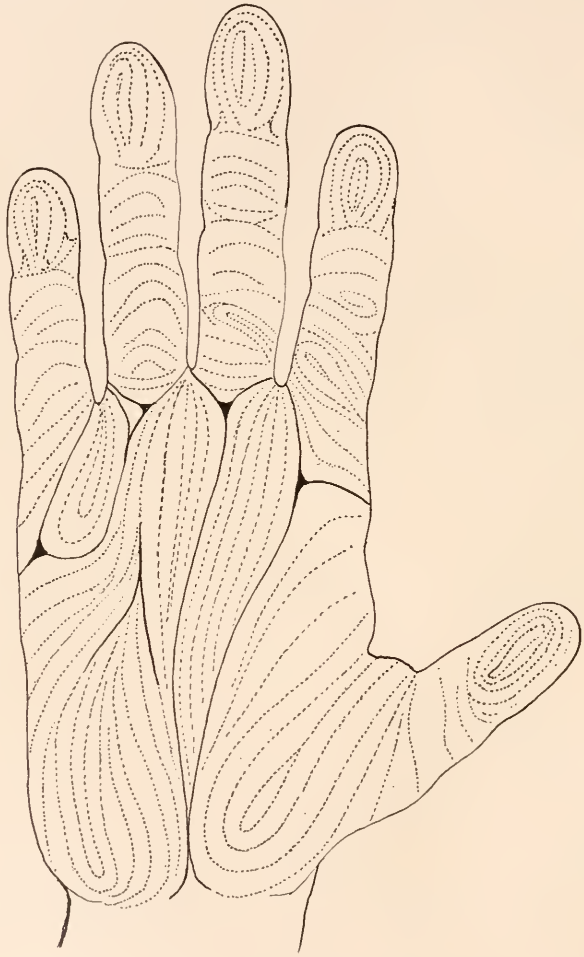


FIG. 11. Hand of a chimpanzee (*Anthropopithecus*), taken from a drawing by W. Kidd, and treated in the same way as Fig. 10, for better comparison with it. Formula: 7·6·2·2.

pass downward in a longitudinal course, and terminate near together in the middle of the wrist. This gives the unheard-of formula 2·2·2·2, if they be considered as terminating together in a carpal triradius, or, what is still more remarkable 1·1·1·1, if they do not.

It is rather unusual, but by no means rare, to find line *A* assuming a low position along the lower third of the free outer border of the palm (position 3); several cases have been met with

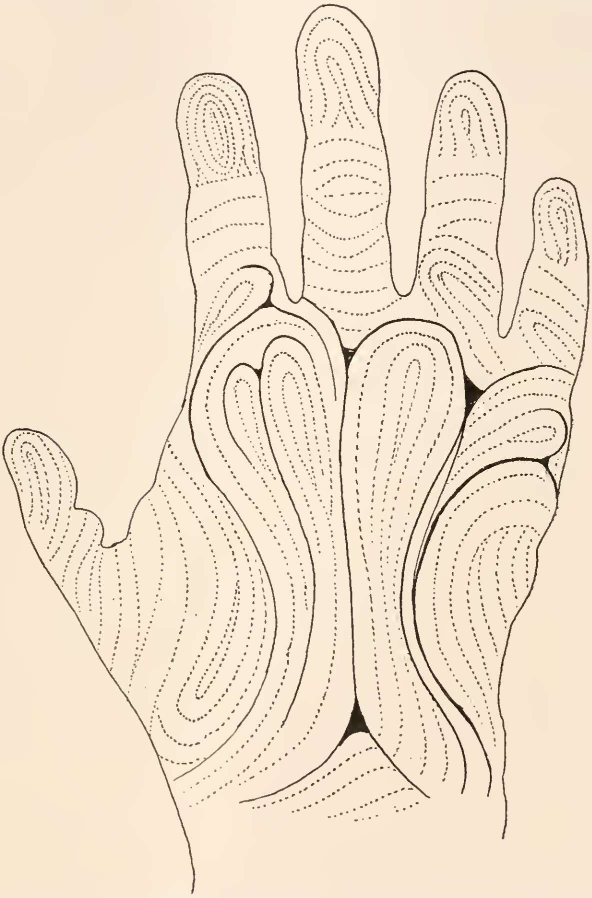


FIG. 12. Hand of a gorilla, taken from a drawing by W. Kidd and treated in the same way as Fig. 10, for better comparison with it. Formula: 3·3·2·1·(?).

in which this line reaches the carpal triradius (2); and one instance is known of line *A* terminating within (on the thumb-side of) this point (position 1); but no such course has ever been recorded for any of the other main lines, not even *B*, which, from its propinquity to *A*, might be thought capable of it. Line *B*, at the lowest position recorded, still keeps within the upper two thirds of the outer (ulnar) margin of the palm, and thus never attains a number less than 5. The known range of line *C* is between 5 and 11, and *D* always curves up, from position 7 on, save in one rather doubtful case in which it appears to curve downwards and outwards, to reach the outer margin at 5.

The right hand of the same individual, while closely similar to the left in general appearance, introduces a slight variation in the form of a lower triradius, which confines a number of the ridges of the third interdigital area, allowing line *C* if one wish to so interpret it, to curve upwards and terminate at position 7, between the ring- and little-fingers. By this interpretation the course of Line *C* becomes quite normal, and suggests the possibility, with a slight rearrangement of lines, of fusing lines *B* and *D*, giving the total formula 10·7·6·1, which, except for the position of line *A*, is not at all unusual.

This right hand, much like the left but with the profound change produced by the presence of the lower triradius in the third interdigital area, suggests a partial explanation for the wholly anomalous condition of the other side, for in the left palm, at the point corresponding to the lower triradius of the right there seem to be one or two ridges that curve and suggest the last vestiges of a vanished triradius like that of the right. The presence of such a formation in this place might bring about a fusion of lines *C* and *D*, making the first two terms of the formula 6·8, yet even thus lines *A* and *B* are not explained, and remain wholly abnormal.

Whatever the explanation, the picture presented by these two palm prints, especially the left, with the ridges crossing the hand lengthwise, is strikingly like that exhibited by the large Anthropoids. This is shown in Figs. 11 and 12, put into the form of diagrams after the sketches of Kidd (1907; Figs. 48 and 46). Since, however, the figures of this author were drawings from the objects, and not print impressions, and as they were designed to show the relief, including certain of the deepest rugæ, it was often difficult to determine the exact course of the ridges, and they are not wholly trustworthy on that account. The similarity of these to the case here presented is obvious; they may even be formulated by the method devised for human palms, the formula being, respectively 7·6·2·2 and 3·3·2·1.

The family to which the subject of this sketch belongs, No. 647 of my collection, is of good old English ancestry, and numbers among her near relatives several persons of more than ordinary distinction. The subject herself is an attractive young person of excellent mind, graduating from college with distinction, and having nothing at all abnormal about her, save this singular arrangement of the palmar friction-ridges.

An investigation of the hand prints of both parents, revealed typical European racial characters, such as the formula 11·9·7·5 in the right hand of the father and the left of the mother; the father's left hand bears the formula 11·7·7·4, and the mother's right is 11·11·9·5, a little unusual but quite normal for a white person.

Unfortunately, owing to the prejudice of the family, I was not permitted to obtain sole-prints of either the subject herself or of her parents, and consequently can make no further report.

III. The Border Region of the Plantar Friction-Skin.

Unlike the condition in the hand, where the friction-skin of the palm terminates along the borders of the contact surface, the friction-skin of the foot extends up along its sides considerably beyond the physiological sole. Since friction-skin has little or no pigment, this extension upwards gives the feet of dark-skinned races, when barefooted, the well-known appearance of pink slippers worn over black stockings.

It thus happens that, while in the hand a simple contact print includes practically the entire friction-skin area, a similar print of the foot includes only the inner portion of the ridged skin, leaving an appreciable border entirely around the part printed, concerning which no record is made. The relation of this *tread-area*, the usual extent of a print, to the entire surface covered with friction-skin is shown in a few examples here, and convinces one immediately of the insufficiency of a tread-area print (Figs. 13 and 14).

The most common loss is that of the loop upon the outer edge of the sole, the probable equivalent of the hypothenar, or of a part of it. This loop is extremely frequent, but as its center occurs near the hypothenar edge, it is more than likely to fall just beyond the edge of a tread-area print. Thus, when completely printed, Figs. 13 and 15 are alike in respect to the loop in question, but with ordinary tread area prints the loop in the former would escape observation. In Fig. 16 a single hypothenar loop would be evident in a tread-area print, but it would never be mistrusted

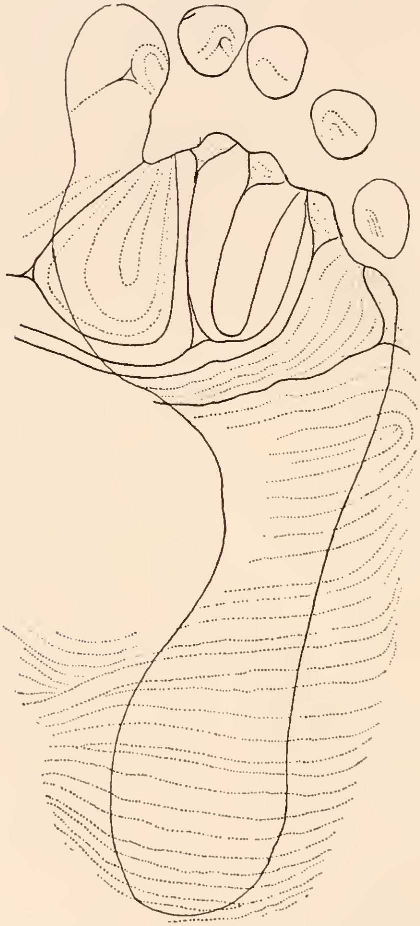


FIG. 13. Print of right foot of Coll. No. 22, showing hypothenar loop, and thenar rudiment, both outside of the tread area.

that there was a second loop proximal to the other, or that the two were separated by a triradius; in Fig. 17, closely similar to 16, a tread-area print would show simply an uninteresting sole, devoid of all special features, and would fail to show either hypothenar loop or the triradius between them.

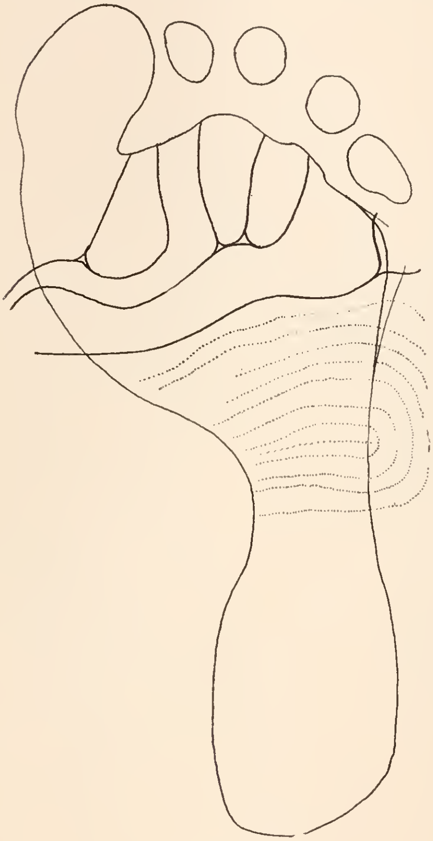


Fig. 14.

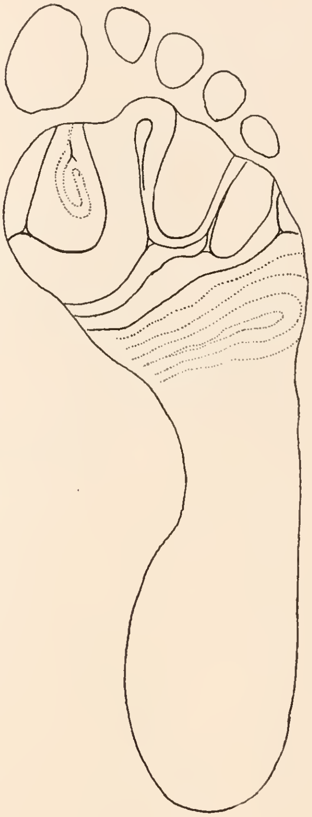


Fig. 15.

Fig. 14. Print of right foot of Coll. No. 202, showing extensive hypothenar loop, not shown by an ordinary tread area print.

Fig. 15. Print of right foot of Coll. No. 609, showing hypothenar loop, practically identical with that of Fig. 13, but so placed that it appears in a tread area print.

Thus far, in the history of the investigation of human soles, there have been on record but three cases in which the general surface of the sole, proximal to the ball of the foot, shows a pattern—a West African negro,[1] a Pole,[2] and a Liberian negro.[3] For the sake of comparison they are all reproduced here (Figs. 18, 19, 20), that of the Liberian being completed in theory

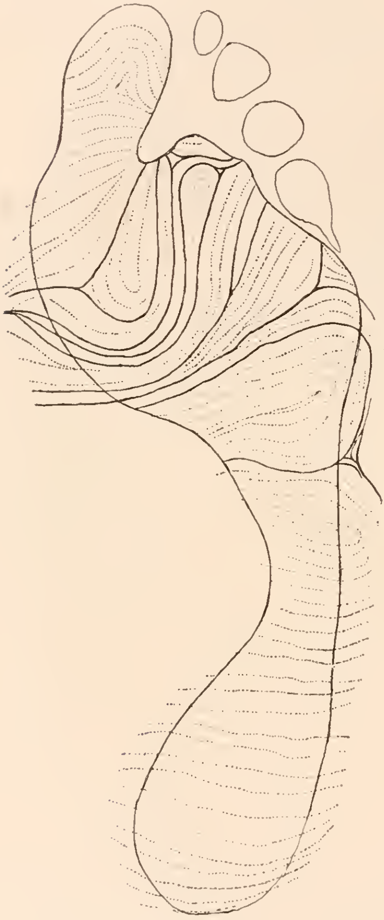


FIG. 16.

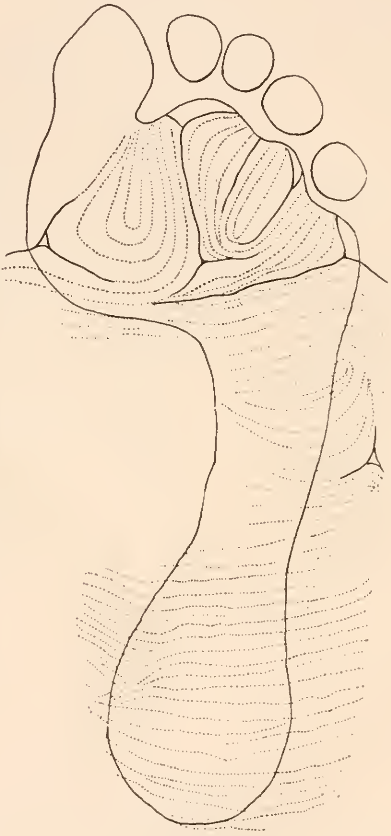


FIG. 17.

FIG. 16. Print of right foot of Coll. No. 236, showing two hypothenar loops and a large triradius. An ordinary tread area print would show only the more distal of the two loops, and present a sole, in this particular like Fig. 15.

FIG. 17. Print of the right foot of Coll. No. 183, like Fig. 16, with the addition of a thenar rudiment, the most of which would remain unrevealed by an ordinary print.

[1] Schlaginhaufen, 1905, p. 102.

[2] Mme. Loth, 1912, p. 603.

[3] Wilder, 1913, p. 205.

beyond the limit of the print, to show the probable course of the ridges. Now in all three cases we are dealing with loops that run across the sole, and in any one of them, if the core of the loop

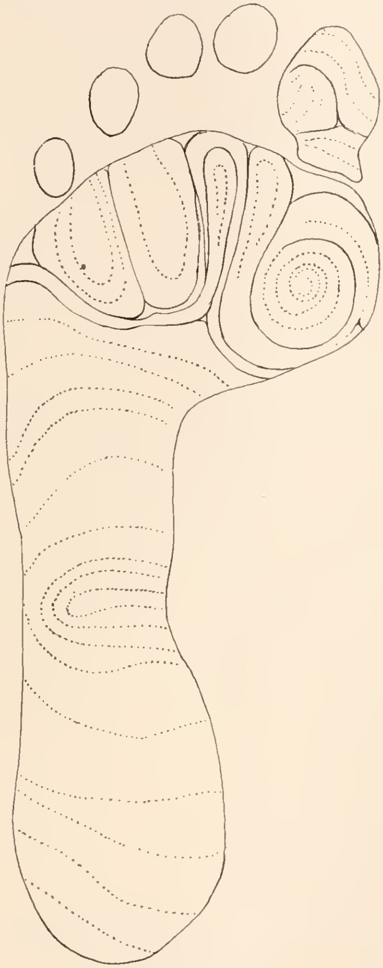


FIG. 18.

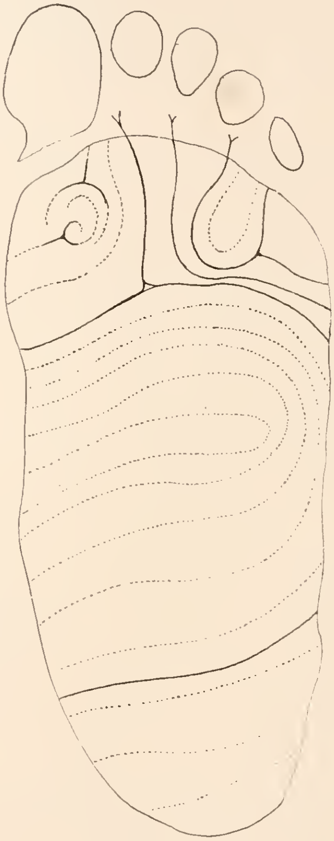


FIG. 19.

FIG. 18. Print of Schlaginhaufen's case; a West African negro, with a loop in the hollow in the foot. Both feet were practically the same. Compare with Fig. 14.
FIG. 19. Mme Loth's case; a negro from North America. Compare with Fig. 14.

had chanced to have been placed a little more laterally there would have been shown simply a sole crossed by the usual transverse lines. Even as it is, in two of the three cases, Mme.

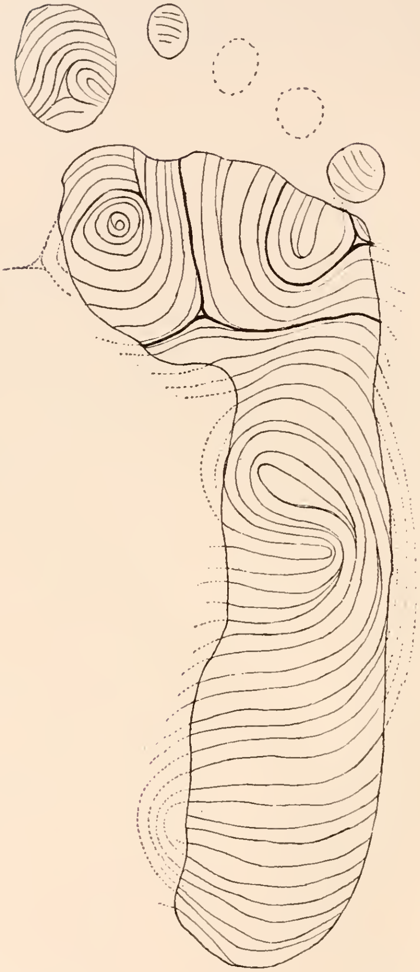


FIG. 20. Print of right foot of Liberian negro; H. H. W. Coll. No. 585, collected by F. Starr. Here the loop which corresponds with the two preceding is brought into association with a more distal one turned the other way, and perhaps, if we may believe in the theoretical completion of the figure as indicated, there is involved a third loop, more proximal than the others. Careful notice must be taken that the heavy lines of the margin, which indicate the tread area, are here all that we have, as the subject is somewhere in Liberia, and not available, and therefore, that the completion of the lines outside of this margin are wholly theoretical. In all of the other cases the lines outside of the tread area rest upon actual prints obtained by rolling the foot. This print has been previously printed (*Amer. Anthropol.*, 1913, p. 205).

Loth's and mine, the individuals were flat-footed and the prints were broader than usual, so that if the feet had had the customary height of arch these figures would have lacked in extent, and in the case of the Liberian the loop upon the inner side might have been entirely lost.

It thus forcibly suggests itself that such patterns as the three just considered *may not be as rare as has been thought, but that many of the prints of the usual monotonous type, consisting, through the middle of the foot, of transverse parallel lines without special features, might yield interesting results if the entire friction-skin area were included.*

Some years ago I attempted to remedy this defect by rolling the foot while being printed, first to the outer, and then to the inner, side, but while this proved fairly satisfactory for the outer portion, the hollow part of the sole, upon the inner side, still remained unprinted, and left a large oval area without record. Schlaginhaufen also, who devoted some study to this hollow region in his extensive work on the Planta (1905) has later sought to include in his records these neglected parts, and has places in his printed record sheet for (1) the tread area, (2) the rolled outer edge, (3) the inner hollow of the foot, and (4) the back of the heel, thus making the record a complete one (1912).

In my own attempts to investigate this border area of the soles I find it expedient, first, to make a careful study of the foot itself with a lens, and then to prepare prints corresponding in general to those recommended by Schlaginhaufen; but in some cases I supplement these by strips, applied to the inked foot, and extending from one triradius, or other feature, to another, in order that they may be correctly oriented. Naturally, the three-dimensional object which the friction-skin covers makes it impossible to reduce the whole to an exact plane, but by overlapping the strips, superposing them carefully at the triradii, pattern-cores, and other important places, and then tracing the whole by a thin piece of paper which covers it, such a figure as that shown here (Fig. 21) may be obtained, which is approximately correct. For the details of pattern-cores, etc., the separate slips are sufficient, but the exact orientation of these must naturally be preserved.

Prints of the tread-area and of the rolled outer edge are obtained in the usual way, by the use of an inked surface, upon which the part to be printed is first placed, and then placed, in the same way, upon a piece of white paper; but the strips used for details, or those covering curved surfaces, are best printed by first inking

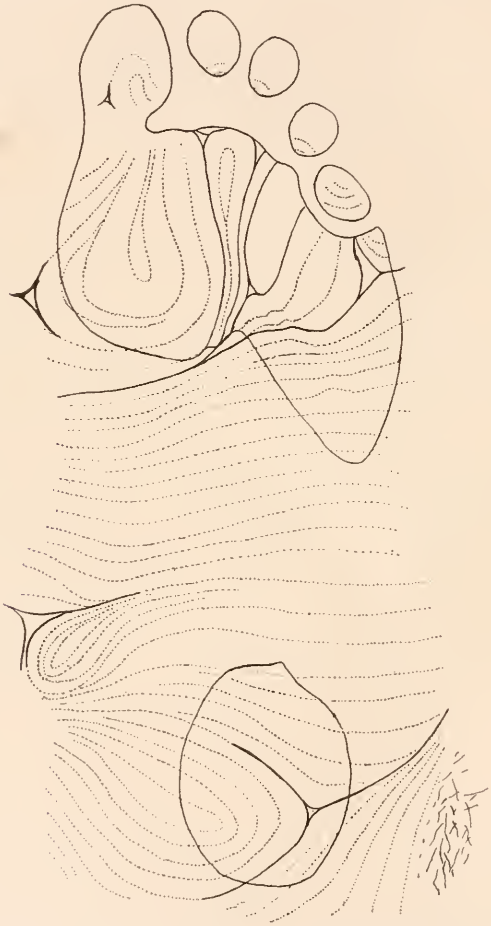


FIG. 21. Print of right foot of Coll. No. 87, reconstructed from impressions taken of various areas. Here the arch is very high and the tread area, indicated by the entire line, is very inadequate. This is one of the few cases known which show a *calcar* pattern, here, as elsewhere, consisting of a single loop on the heel, open to the tibial side, and slanted somewhat distally, thus coming into close relation with the *thenar* pattern, covering the small thenar eminence. This latter pattern is also in the form of a loop, with its opening directed fibulo-distally, and is supplied with a triradius.

the foot and then putting on the slips, applied something like bandages, and pressed with the hand. To ink the foot the roller used in inking the paper surface may be employed, care being taken to spread the ink evenly and not too thickly. The applied strip may be pressed or gently rubbed with the hand.

One of the chief gains resulting from the study of the completed friction-skin of the foot lies in the added material furnished for the study of the proximal row of patterns, the *thenar* and the

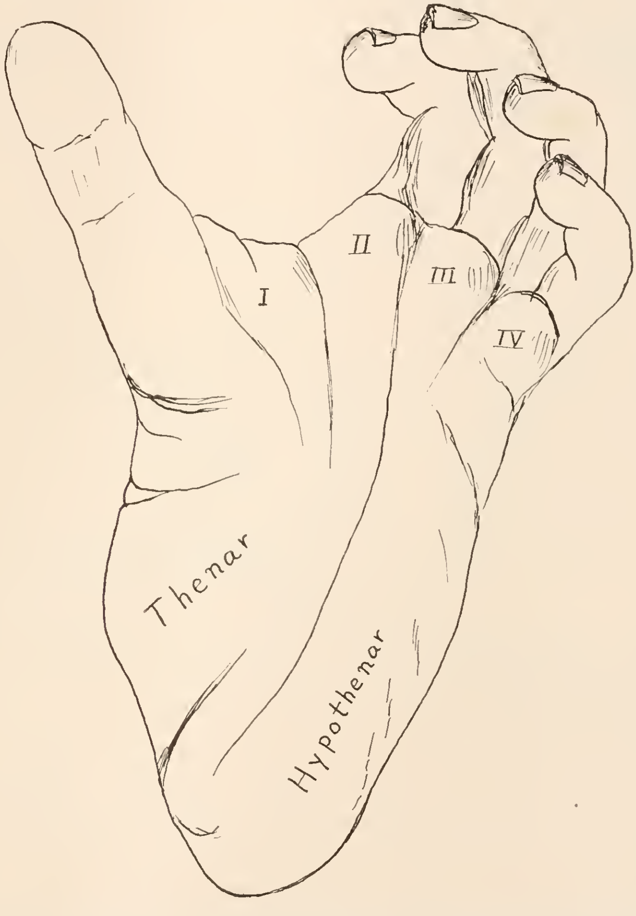


FIG. 22. Drawing of the plantar aspect of the right foot of a chimpanzee, to show the normal position and relations of the four interdigital and the two proximal pads. For comparison with Fig. 23.

hypothenar. The long, extended heel of the human foot is so unlike anything found in typical mammals that even the location in the human subject of these once important eminences, with their associated patterns, is by no means a simple matter. The best aid here comes from the comparison of the human foot with that of some one of the large Anthropoids, preferably a Chimpanzee (Fig. 22), where both the thenar and hypothenar are still evident, although the drawing-out process has already commenced. The backward extension seems here to involve mainly the hypothenar region, and as this surmise is supported by the relation of the underlying skeletal parts, we may look upon *the entire human sole,* back of the "ball of the foot," *i. e.,* the interdigital pads, *as an extended hypothenar.* The thenar seems to take no part in the extension, but lies passively upon the medial

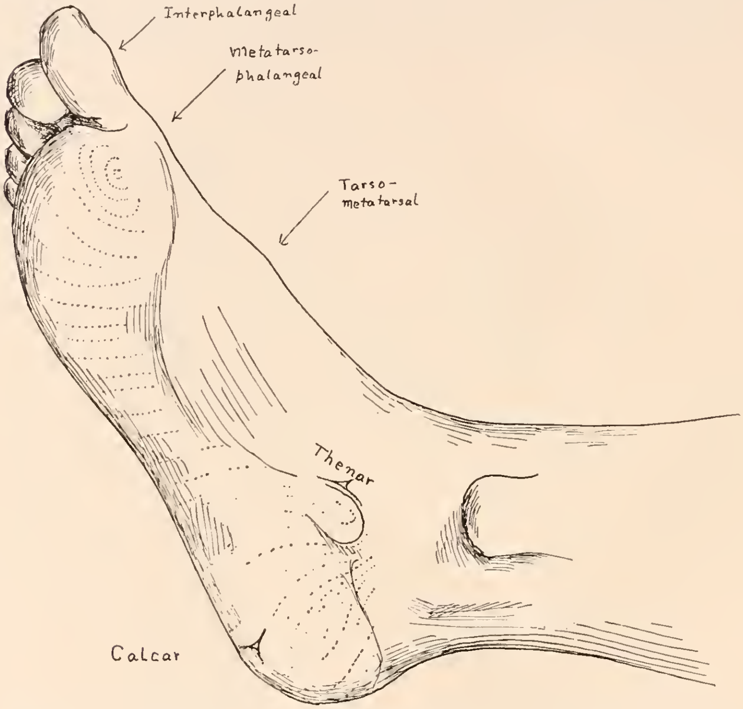


FIG. 23. Diagrammatic drawing of a right human foot, resting upon the heel, as seen in a recumbent figure; inner (tibial) aspect. The thenar pad is exaggerated, but accurately located. For comparison with Fig. 22.

side of the foot, quite above the tread area, where in man it may still be found greatly reduced in size and importance, but occasionally quite evident (Fig. 23).

Difficult to even locate at first, one soon becomes accustomed to picking out the thenar pad on almost any foot, and in cases where it is really prominent, and when seen in the right light, it forms a conspicuous object. The friction-ridges crossing this eminence almost always exhibit some disturbance of their otherwise even course across the foot, and occasionally show a distant loop with a triradius (Fig. 21). As is to be expected, too, the best developed patterns are likely to occur on the most conspicuous pads, the atavistic tendency manifesting itself in these two ways at the same time. The last vestige of the thenar pattern is indicated upon a flat surface, without trace of a pad, by a slight disturbance of the friction-ridges in this place (Figs. 13, 17, 24, and 25).

Miss Whipple, in her fundamental work upon the whole subject of epidermic ridges, has considered the causes of the degeneration of the thenar pad of man and finds the principal one in the establishment of the long arch, spanning the distance between the ball and the heel. The thenar pad, and, as she states, the hypothenar also, are both situated directly beneath the center of the long arch, and their reduction thus becomes a matter of necessity.[1] Schlaginhaufen[2] gives several prints of the thenar pattern, without definitely designating it as such, and was quite right in stating that he was the first to carefully investigate this pattern. In its best developed form this thenar pattern, as thus far recorded, seems to consist of a simple loop with a single triradius (Fig. 21), or occasionally, two loops, with the triradius between them.[3]

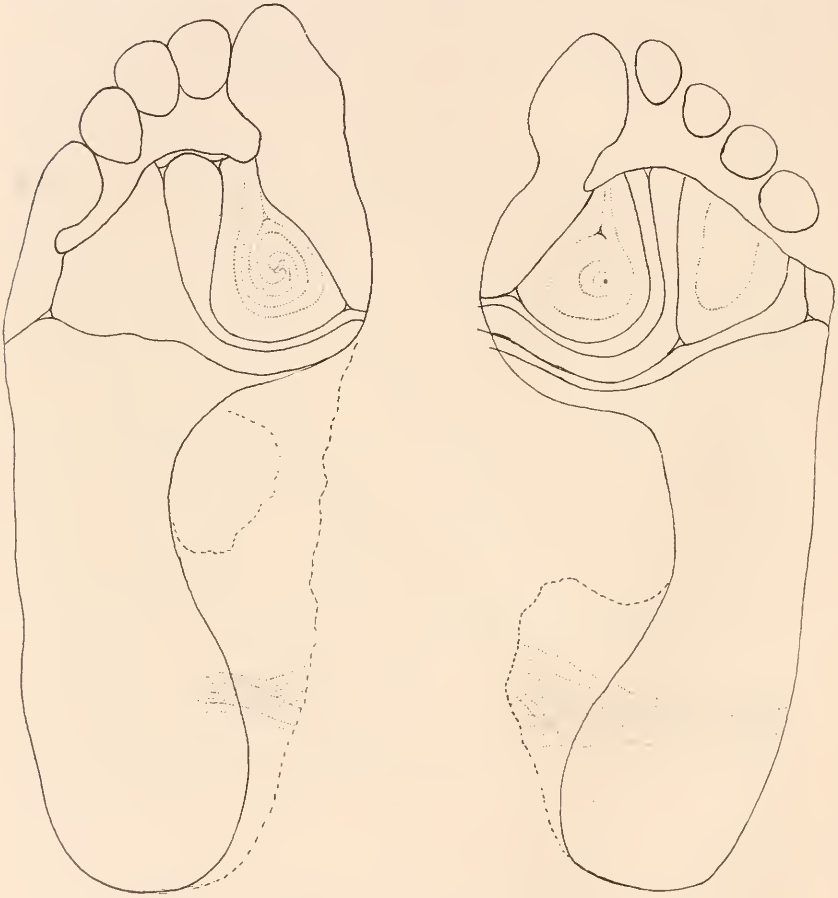
Light is shed upon the question of the hypothenar pattern by the interpretation of the long-extended human sole as formed from the hypothenar region, since, if this be true, the pattern, or elements of it, may thus be looked for on any part of this area. This would determine at once as hypothenar, not only the narrow loop found quite commonly upon the fibular edge of the foot, a little proximal to the ball (Figs. 13, 14, 15) but also the second loop further back (Figs. 16 and 17), and probably also the enormous figure found in the middle of the arch in the three special cases mentioned (Figs. 18, 19, 20). Aside from these three elements there is also the *calcar* pattern, which, aside from a single family, in which it occurs in father, mother and two of the three children, I have seen but twice. (See below, Part VI.)

---

[1] Cf. Miss Whipple, 1904, p. 292, Fig. 19.

[2] 1905, pp. 107–109, Figs. 182–184.

[3] Schlaginhaufen, *loc. cit.*, Fig. 183, 1.



FIGS. 24 and 25. Prints of the two soles of the same individual (Coll. No. 199), in which an inward rolling of the feet reveals on both sides a rudimentary thenar pattern.

Now, at the present state of our knowledge it is impossible to say whether all of these patterns, or pattern fragments, are parts of a single hypothenar pattern, which has become spread out, and its parts disassociated, by the great extension of the part covered by it, or whether there are added to the genuine hypothenar elements certain "secondary patterns" (Whipple), like those covering the proximal and medial phalangeal surfaces in certain apes. The *calcar* pattern is treated as a secondary one by Miss Whipple,[1] and is figured in two specimens of *Cebus*, where it appears definitely distinct from thenar and hypothenar, although in contact with both.[2] On the other hand it involves no improbability to treat all of these elements, with the possible exceptions of the calcar, as the more or less disassociated parts of a long-drawn-out hypothenar, the result of a great extension of its field in one direction and finds a close analogy in the apical patterns of the four lesser toes in the human foot, where the pattern is drawn out laterally to such an extent that there occur not only long extended S-shaped figures, with the two loops far apart, but even those with three loops and three associated triradii. One of the latter is figured by Schlaginhaufen,[3] and the same type is described briefly by Miss Whipple,[4] where "the pattern has become separated into distinct loops and an accessory degeneration triradius is introduced, that is, a triradius not originally present in the typical scheme but formed incidentally in the process of degeneration of the pattern."

It is thus *a priori* probable that the three elements represented by (1) the simple hypothenar loop of the fibular side (Fig. 13), (2) the second loop occasionally present proximal to the latter (Fig. 16), and (3) the large loop in the middle of the foot, of which but three instances have thus far been described (Figs. 18, 19 and 20), are all parts of a degenerated hypothenar, yet the homologies of these several elements with one another, and the course of degeneration in the original hypothenar pattern, with the interpretation of these various existing vestiges, is still a large problem. For this there is great need of more data, complete prints of the soles of a large number of individuals, each one including the border areas of the friction-skin, with the portions so taken that recognizable features of the usual tread area are included, to allow definite orientation.

---

[1] 1904, p. 361; Taf. VI.

[2] P. 334, Fig. 37, *b* and *c*.

[3] 1905, p. 114, Fig. 185.

[4] 1904, pp. 352–353.

From the few sole prints given here there are certain deductions and surmises concerning these possible hypothenar elements, that are at once apparent, and may be here mentioned, rather as suggestions to stimulate comparison than as definite assertions.

1. All the elements here considered are loops, and, with the exception of one of the loops of Fig. 20, all open toward the medial, or tibial, margin of the sole. It is thus difficult to explain them as the two disassociated ends of a long S-shaped figure.

2. In the case of Fig. 16, with two large loops facing the same way, the more distal is probably the loop commonly found on the outer margin, near the ball of the foot, while the other is readily comparable with the large loop in the hollow of the foot, as given in the cases of Schlaginhaufen and Mme. Loth (Figs. 18 and 19). If, however, we compare Fig. 16 with Fig. 17, we see this same proximal loop gradually reduced to a small but evident figure, on the way towards extinction. This gives us a series, Figs. 19, 18, 16 and 17, in which a loop begins with occupying almost the entire sole, and ends as a rudiment. One recalls here Schlaginhaufen's interesting series, in part theoretical, in which he derives such a case as that of Mme. Loth, from a moderate-sized loop, found in lower Primates, which crosses the heel region obliquely, and opens to the medial side (Schlaginhaufen, loc. cit., p. 121).

3. Concerning the calcar pattern, about which so little is known, a pattern which has been observed here and there, but has as yet no morphological interpretation, I made recently more careful studies upon No. 87 of my collection, a subject who has a good calcar pattern upon each heel. The result of this, in the case of the right foot, where there is also a good thenar pattern, is given in Fig. 21 above. In the figure the whole record of the friction-skin of the foot is spread out as reduced to a plane, and the tread area is indicated by lines. Unfortunately there are no indications of the loops here considered hypothenar, so that a comparison of either thenar or calcar with these is not possible; *on the other hand there is revealed a possible association of calcar with thenar, suggesting that the two form the two loops of an S-shaped pattern.* This close association of thenar and calcar patterns is not new, being shown by Miss Whipple in two speci-

mens of *Cebus*[1] and in *Inuus*,[2] but here the fields occupied by the two patterns seem more distinct. This author treats the calcar pattern as a secondary one, formed probably through the backward extension of the heel, to assist in covering the new area, and sees a proof of its recent nature from the fact that there is no trace of such a pattern in the corresponding position in *Lagothrix*, where "the calcar region is still covered by epidermic elements not yet fused into ridges."

An element to be considered in this connection, but one which adds to the confusion rather than assists in the elucidation, is the "Fersen-sinus" of Schlaginhaufen, which he figures on the heel of various Primates, the core of which usually forms a loop opening to the medial side, as in man. This he shows most typically in *Macacus* and *Hylobates*, but it appears also in *Simia*, *Gorilla*, and *Anthropopithecus*. As this author does not emphasize the homologies of the typical patterns as located upon the original pads, but compares rather the triradii and lines proceeding from them, one can hardly follow the patterns through his numerous figures.

IV. THE REDUCTION OF LINE *C*.

In 1910 Edward Loth, assisted by Mme. Loth (Jadwiga Niemirycz-Lothowa), published the results of the examination of a large collection of the palm and sole prints of Russian Poles from the vicinity of Warsaw. In these they find a number of instances in which line *C*, with its triradius, is entirely wanting, and others in which the line in question is very short, and terminates, after a perfectly straight course, in a loop (cf. Figs. 33 and 34 below). These conditions, which are obviously closely related, both designates in a main line formula by the letter x.

With regard to previous recognition of either of these two conditions he cites Miss Whipple in her paper of 1904, and states very generously that he makes no claim to priority, yet thinks that he is the first to indicate it in formulæ.

As this condition has been so long known to me, practically from the beginning of my investigations, I felt sure that I had described it in detail somewhere, but, to my own chagrin, I can find no direct reference to it in any of my writings. I have indicated this condition in formulæ, however, by the digit 8, the designation for the radius of origin of the missing line, intending to signify by this that it goes nowhere, yet I am quite ready to acknowledge that the meaning of this is not clear, and that Loth's use of the letter x is much clearer.

---

[1] *Loc. cit.*, p. 334.

[2] P. 307.

Since the condition itself is an interesting one, I have recently taken some pains to estimate its frequency. In the hands of 145 white persons (mostly Smith College students) a complete loss of line *C*, with its triradius, was found in both hands in four cases; in the right alone in five; and in the left alone in eight. That is, out of 145 individuals, no less than 17 of them showed a complete loss of the *C* line in one or both hands; or, put another way, out of 290 separate hands 21 were thus marked. In addition to these, nine more individuals possessed in one hand a very short and straight *C* line, ending in a loop, and as these were none of them duplicate individuals with any of the above or with each other, this gives a total of 26 individuals out of the 145 in which the term x (= 8) occurs in one or both of the hand formulæ as a designation of the condition of line *C*; that is, nearly 18 per cent.

Comparing the palms of races other than white I found eight cases in 42 palms of the Maya Indians of Yucatan, or 19 per cent. much as in the whites, while in 118 palms of Liberian soldiers this condition (including both forms of it) occured but 10 times, 11.8 per cent.

To summarize these results: the condition of line *C*, in which it is either wholly absent, together with its triradius, or very short and ends in a loop, is a fairly common one, apparently in all races. In the negro palm, however, the marked tendency of all the lines to run diagonally across the palm, from the bases of the fingers to a more proximal position on the ulnar margin, which results so commonly in the formula 7·5·5·5, considerably lessens the percentage of occurrence of this condition.

In expressing this condition in a formula I would suggest the general adoption of Loth's abbreviation, x, when the line and triradius are both wanting, and that of 8, my usage hitherto, to designate a very short *C* line, ending in a loop.

(*To be continued.*)

HEREDITY AND ORGANIC SYMMETRY IN ARMADILLO QUADRUPLETS.

II. Mode of Inheritance of Double Scutes and a Discussion of Organic Symmetry.

H. H. NEWMAN.

A. General Statement.

This paper is in continuation of a study published under the same general title in July, 1915. In that paper the inheritance and distribution among quadruplets of more or less extensive band anomalies were dealt with. All anomalies involving the presence of two or more consecutive double scutes either in mother or in one or more offspring of a polyembryonic set were considered as *band* anomalies. There remained numerous cases of inherited anomalies so minute as to involve only isolated double scutes in parent and in offspring. A detailed study of the inheritance and of the symmetrical or asymmetrical distribution of these double scutes among the fetuses of quadruplet sets forms the subject matter of the present contribution.

In order to have a compact and truly homogeneous group to work with, I have decided to confine my study to collections *C* and *K*, omitting several small collections, the data of which are not so complete. In the *C* and *K* collection there are 140 sets of quadruplets which are sufficiently advanced to show every detail of the scute pattern. The shells of the mothers of all these sets are preserved and have been carefully scutinized for anomalies. Such shells as were badly worn or damaged had to be excluded from the present study together with the associated offspring. A study of 140 sets should reveal the conditions typical for the species, since we have a collection of 700 individuals. Of the 140 sets of quadruplets 73 are female and 67 male. This discrepancy between the sexes is due to two circumstances, first that three sets of fetuses, in which the sex was obviously male, were too small to count, the mothers in all cases being normal, and second that two sets of offspring, all normal and males, were discarded because the shell of the mother in each case was badly damaged. If these five sets of male quadruplets be added the count would be practically even so far as sex goes, 73 females and 72 males. Other collections, moreover, have shown equal numbers of male and female sets of quadruplets.

1. *Three Categories of Offspring.*

A survey of the 140 sets of quadruplets reveals that there are three well-defined classes:

(*a*) Those in which both mother and offspring have anomalies. Of these there are 56 sets, 29 female and 27 male.

(*b*) Those in which the mother is normal but the offspring have anomalies. Of these there are 41 sets, of which 22 are female and 19 male.

(*c*) Those in which both mother and offspring are normal. Of these there are 43 sets, of which 22 are female and 21 male.

Unless the character in question is strongly inherited as a dominant we would expect to find a fourth class composed of sets in which the mother has an anomaly but all offspring are normal. Only one such case occurs, and this is a doubtful one in which the anomaly of the mother is a double scute that may be due to the fusion of two scutes after a local injury. This doubtful case has been included in class (*c*). Another case that I at first thought was in this fourth class was found on examination to belong to class (*a*), for one of the fetuses had a double scute in the last scapular band which had been overlooked in the counts because we were dealing only with the regular bands. The facts of this case will be clear from an examination of Set *C* 65 (bottom of *p.* 187).

The mode of inheritance of these anomalies does not appear to be typically Mendelian, for, if the character is a dominant, with the normal condition the recessive, we would expect a considerable number of anomalous individuals to be heterozygous for the character and to produce equal numbers of germ cells with the anomaly factor and without it; so that on the basis of chance mating we should often get normal offspring from the mating of two heterozygous anomalous parents or from one heterozygous anomalous parent pairing with a normal parent. That we do not find this condition means that we have evidently a non-Mendelian result due probably to factor segregation among the quadruplets, a process that must evidently take place in order to produce sets in which some individuals are anomalous and some are normal. This segregation must also affect the germ cells, so that an individual that has an anomaly has also germ cells of only one kind and these homozygous for the anomaly factor. The same kind of mechanism that quite evidently segregates the somatic anomaly factor must also be conceived of as responsible for a segregation of the germinal anomaly factor. The results do not appear to bear any other interpretation. Further discussion of this point follows the presentation of data.

Since these anomalies are invariably inherited when present in the mother we are driven to the conclusion that all these sets in class (*b*), which have anomalies, but whose mothers are normal, must have inherited their anomalies from the father. Furthermore, it is equally obvious that a considerable proportion of those sets in class (*a*) must have anomalous fathers as well as anomalous mothers. Thus class (*a*) is heterogeneous in that it is composed of sets inheriting from both parents, on the one hand, and from the mother alone, on the other.

A calculation may readily be made of the relative sizes of the various classes of offspring that should result if chance mating occurs between anomalous and normal individuals. It will be noted that 56 of the 140 mothers (or 40 per cent.) have anomalies. Again 115 individual female offspring, or 39+ per cent. and 111 individual male offspring or 41+ per cent., have anomalies. So we may be safe then in assuming that 40 per cent. of the individuals of the species have anomalies and 60 per cent. have none. If 140 chance matings on this basis occur we should expect the following classes:

(*a*) 16 per cent. or 22.4 with both parents anomalous.
(*a*′) 24 per cent. or 33.6 with mother only anomalous.
(*b*) 24 per cent. or 33.6 with father only anomalous.
(*c*) 36 per cent. or 50.4 with neither parent anomalous.

The sum of classes (*a*) and (*a*′) is 56, which is exactly the number of sets in the observed class (*a*).

The observed class (*b*), 41 sets, is several sets in excess of expectation, but not enough to seriously effect the theory of breeding proposed. The observed class (*c*), 43 sets, is several sets too low, but it is very probable that the five discarded male sets referred to above belong to this category. On the whole, then, the observed and theoretical proportions of the categories of offspring are as close as could be expected in 140 matings, and this fact supports the general statement as to the modes of inheritance of these anomalies stated above.

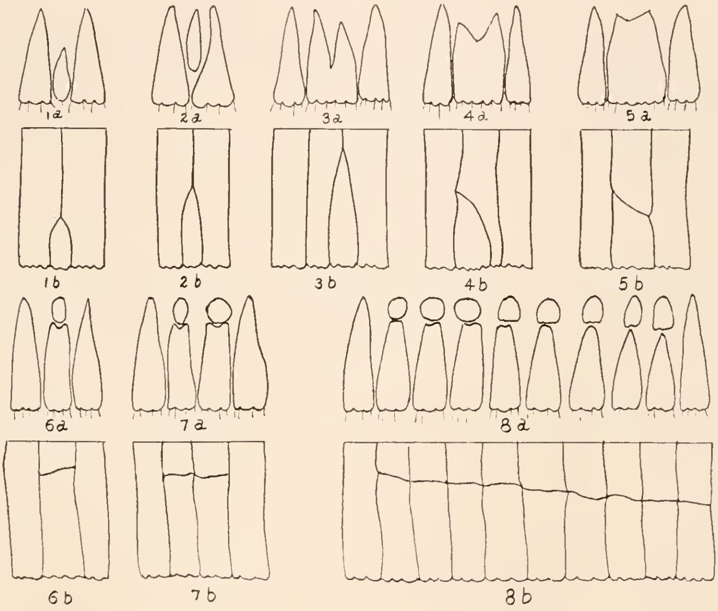
2. *The Genetic Relation Between Scute and Band Anomalies.*

There is a very intimate genetic relationship between band and scute anomalies, as was brought out in the previous paper (Newman, '15). Sometimes a band anomaly is inherited as such in some offspring of a set, and as a scute anomaly in others; and the localization in all cases is so exact that there can be no doubt about the genetic equivalence of the two anomaly phases.

In Figs. 1–8 is arranged a series of drawings of scute anomalies leading up to band anomalies. The transition is more readily made out from the conditions in the bony plates underlying the scutes. Fig. 1*a* represents the appearance from the exterior where only the scute is visible; Fig. 1*b* shows the underlying plate condition. Similarly with Figs. 2*a* and 2*b*, etc. In Fig. 6, *a* and *b*, we see that the more fundamental anomaly is a transverse splitting of the bony plate involving only one unit. Fig. 7, *a* and *b*, constitutes an incipient or minimal double band, while Fig. 8, *a* and *b*, represents a band doubling of moderate extent involving six scutes. Such band doublings may involve more than half of a band or there may be extensive doubled regions separated by single or undoubled regions.

Since the bony plates are fully laid down only during post-embryonic life, it is impossible to study these structures in the fetuses that are taken from the uteri of the mothers; but the scute condition, for one who has made a study of the subject, serves as a very definite index of the condition of the bony plate; and the scutes are well defined long before birth. For example, whenever a scute is found with a deep notch above dividing the scute into two nearly equal cusps (as in Fig. 3*a*), one can be sure that the bony condition will be like that in 3*b*. Similarly when the notch is shallow as in 5*a*, we might expect the bony condition to be as in 5*b*.

It is an open question as to which doubling is more fundamental, that of the dermal plate or that of the horny scute. Ontogetically the scute is the older structure, but I am inclined to look upon the two structures as phylogenetically coeval, for we apparently have in the armadillo a case of the persistence in a mammal of the ancestral reptilian epidermal scale with its dermal bony core. The hair group that centers about the scute is presumably of more recent origin.



Figs. 1–8.

I look upon scute and plate doubling as cases of local budding of normally single primordia, not unlike the division of scutes and plates in the carapace of modern tortoises, a phenomenon that has received considerable attention (Newman, '05, and Coker, '10). The physiological basis of this budding may be a localized lowering of the rate of growth during scute development. If we had a solution of the problem of budding or division in this instance we would have a solution of the general problem of division that appears everywhere in the field of biology.

Since the inheritance and distribution of double bands was dealt with in detail in the earlier paper of this series it will not be necessary to refer further to these results. It may be of interest, however, to compare the facts as to symmetry phenomena in the two classes of anomaly. Band anomalies are much less numerous than anomalies of single scutes, but they exhibit the same mirror-image phenomena and symmetry reversals that are so frequently seen for double scutes. For details of these phenomena the reader is referred to the published account (Newman, '15).

The present study deals solely with scute anomalies, double or split scutes of the various types shown in Figs. 1 to 6.

3. *Frequency and Distribution of Scute Anomalies.*

In the present collections (*C* and *K*) there are 700 individuals composed of 140 sets of quadruplets and their mothers. These should constitute for statistical purposes a reasonably adequate sample of the species.

In the earlier paper of this series (Newman, '15) 23 sets that showed band anomalies in one or more members of a set were dealt with. Many of the individuals (40 in all) had scute anomalies, some with and some without accompanying band anomalies. The remaining 117 sets (585 individuals) treated in this paper contain 199 individuals with double scutes. Whether we figure on the basis of the entire collection or upon the 117 sets dealt with in the present paper we find that 34+ per cent. of all individuals have scute anomalies. Add to this 32 (4+ per cent.) individuals that have only band anomalies and we find that about 39 per cent. of all individuals have either band or scute anomalies or both, which is very close to the 40 per cent. arrived at earlier in this paper. Scute anomalies are, however, about eight times as numerous as band anomalies and furnish better material for statistical study.

4. *Sex Distribution of Anomalies.*

Exclusive of the mothers, exactly 40 per cent. of which have anomalies, 22 females and 12 males have band anomalies, and

87 females and 100 males have scute anomalies. There appears therefore to be a somewhat pronounced tendency toward band anomalies as against scute anomalies in females and the reverse in males. In a collection of this size, however, these differences may not be significant.

5. *Distribution of Scute Anomalies as to Bands.*

In the earlier paper of this series it was shown that the band anomalies are largely confined to the first two bands: 86 per cent. in band 1, 8 per cent. in band 2, and the rest scattered; none occurring in bands 5 or 6, which are in the middle of the banded region, or in band 9. The reason given for this state of affairs is that band doubling is normal for the scapular and pelvic regions and that the bands nearest the scapular and pelvic shields are naturally more like these regions than are those farther removed from them.

A somewhat similar condition, though less pronounced, is brought out by a census of double scutes according to bands:

Band 1 has double scutes 67 times.
Band 2 “ “ “ 40 “
Band 3 “ “ “ 40 “
Band 4 “ “ “ 34 “
Band 5 “ “ “ 9 “
Band 6 “ “ “ 27 “
Band 7 “ “ “ 34 “
Band 8 “ “ “ 29 “
Band 9 “ “ “ 9 “

It is significant that double scutes, like double bands, occur most frequently in the first three bands and least frequently in bands 5 and 6 and 9, where band anomalies were entirely absent. Bands 5 and 6 are farthest from the scapular and pelvic regions where doubling is the normal condition. Band 9 is really not a free band at all, but only partially free at the margins; hence it may be left out of consideration. It is really out of the series of bands and should be included in the pelvic shield, but, out of deference to the time honored name "nine-banded armadillo," I have treated it as the ninth band.

I interpret the figures for both double bands and double scutes as I formerly interpreted similar facts brought out by a study of supernumerary scutes in the carapace of tortoises. These supernumerary scutes were viewed as atavistic variations or vestiges of a condition largely outgrown by the species. These anomalies occur most frequently at the posterior end of the carapace and progressively less frequently as one goes toward the anterior end, except that there is a slight increase just at the anterior end. The hypothesis was ventured that the more frequent the regional occurrence of an archaic character the more recent has been the racial suppression of these characters in that region. There has evidently been an antero-posterior orthogenetic loss of scutes in the tortoise carapace beginning at or near the anterior end and ending with the posterior end.

Now in the armadillo I believe that the more generalized and less regular parts of the carapace, the scapular and pelvic shield, represent phylogenetically an older condition than does the banded region. In support of this contention I cite the fact that the extinct giant armadillo, *Glyptodon*, had a solid non-banded carapace. The first banding began in one or two bands at about the middle of the carapace, where bands 5 and 6 are, and proceeded posteriorly and anteriorly. Progress anteriorly in the direction of more bands is probably occurring now, as may be judged by two facts, first that it is not uncommon to find a part of the last scute row of the scapular shield separated as a band, and second that there are not infrequently local fusions between parts of the first band and the scapular shield. On this hypothesis band 1 is the newest band phylogenetically and shows more resemblance to the scapular shield than do other bands, both in band doubling and in scute doubling. Band 2 is much less affected with these anomalies than is band 1, but more so than any other band. Moreover, bands 5 and 6, which are phylogenetically the oldest bands, show the fewest recurrences of irregular conditions. Scute doublings are viewed as incipient or vestigial band doublings and we find many more of these vestigial anomalies in the banded region than fully developed ones.

In the tortoises the orthogenetic progress in carapace simplification has proceeded antero-posteriorly and in this single direction. In the armadillo banding has proceeded both ways from the middle. Various armadillo species today exhibit all the way from one to twelve bands, a fact that supports the idea that in our species there has been a gradual increase in the number of bands.

B. Presentation of Data Concerning the Inheritance And Distribution among Quadruplets of Double Scutes.

The following tabulation gives all of the facts concerning double scutes, except those brought out in connection with the earlier paper on double bands (Newman, '15). I know of no method for presentation of data of this kind that is at once so intelligible and so compact as the pictorial diagram used in this and the former paper. When one has read the key to the table there should be no difficulty in understanding the data thus presented. The facts are first presented for those sets in which both mothers and offspring have double scutes, covering pages 182 to 188, and there follows as an appendix to this paper a table for those sets in which the mother was without double scutes, but in which one or more of the offspring have inherited the double scutes from the unknown father. See pp. 204 to 207.

Key to Tabulation of Double Scutes.

Each solid block of bands shows the anomalies in position as though the bands were straightened and placed with the right-hand end to the right and left-hand to the left. The number of the set with the sex of the quadruplets is indicated above and at the left of each block. Bands of the mother that show anomalies are marked *M*; those of the offspring that show anomalies are marked according to previous custom I., II., III., IV. I. and II. are a natural pair, II. being a primary bud individual and I. its twin secondary bud derivative. Similarly III. and IV. are a natural pair, IV. primary and III. secondary. If the anomaly occurs in different bands of the same individual a bracket includes the Roman numerals indicating the individual fetus concerned. The various types of double scute are drawn in upon the band in as nearly as possible the position in which they occupy, but they take up more space laterally than is actually the case. In addition to positional location the number of each scute, counting from either of the margins or from the middle, is given by an arabic numeral upon the pictured scute, the direction from which the count has proceeded being indicated by an arrow unless this is too obvious to need indication. The arithmetical middle of a band is indicated by a dotted vertical line, except in a few cases where the right and left halves of two different bands of a single individual are placed in the same line, in which case a solid line is used to separate these heterogeneous half bands. This change of method may be a little confusing at first, but it was introduced to save space. The band in which the anomaly occurs is numbered with an arabic numeral followed by a colon and a figure indicating the total number of scutes in that band. For instance 6 : 63 means band 6 which has a total of 63 scutes, counting the double scute as two scutes. L.S. refers to the last scapular row of scutes, next to the bands. This mode of tabulation admits of a very condensed record of a large amount of data.

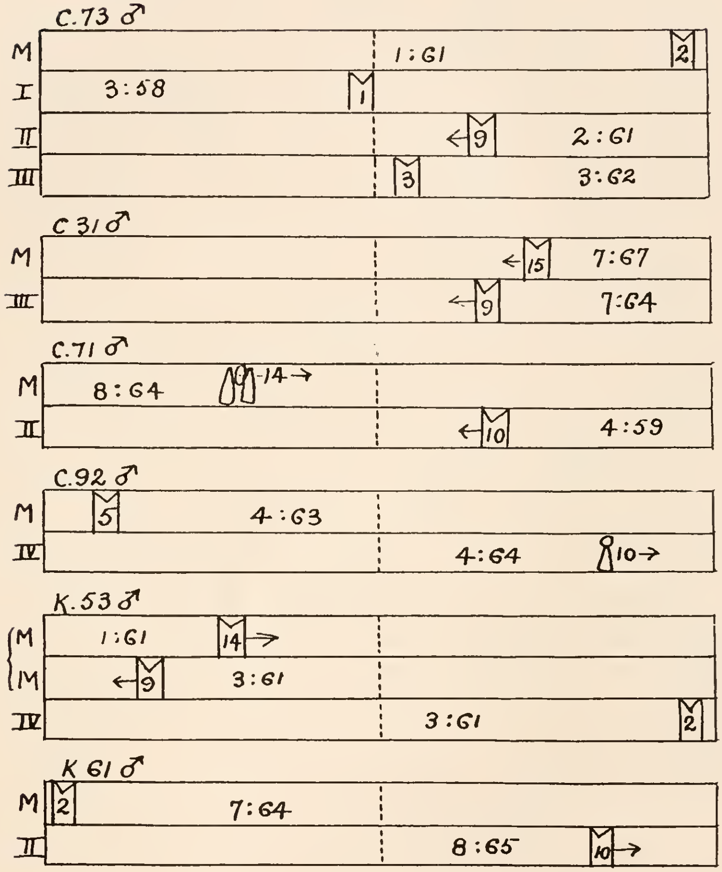

C.73 ♂
M
1:61
2
I
3:58
1
II
9
2:61
III
3
3:62
C 31 ♂
M
15
7:67
III
9
7:64
C.71 ♂
M
8:64
14
II
10
4:59
C.92 ♂
M
5
4:63
IV
4:64
10
K.53 ♂
M
1:61
14
M
9
3:61
IV
3:61
2
K 61 ♂
M
2
7:64
II
8:65
10

Fig. 9.

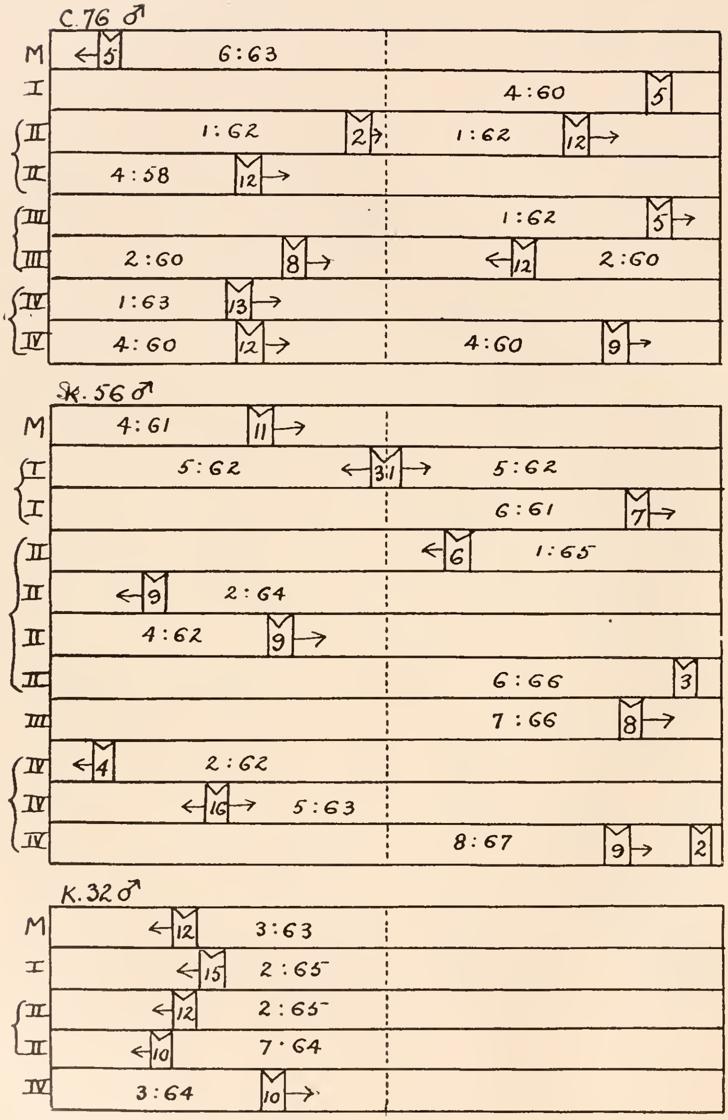

C. 76 ♂
M
I
II
II
III
III
IV
IV
5
6:63
4:60
5
1:62
2
1:62
12
4:58
12
1:62
5
2:60
8
12
2:60
1:63
13
4:60
12
4:60
9
Sk. 56 ♂
M
I
I
II
II
II
II
III
IV
IV
IV
4:61
11
5:62
3.1
5:62
6:61
7
6
1:65
9
2:64
4:62
9
6:66
3
7:66
8
4
2:62
16
5:63
8:67
9
2
K. 32 ♂
M
I
II
II
IV
12
3:63
15
2:65
12
2:65
10
7·64
3:64
10

FIG. 10.

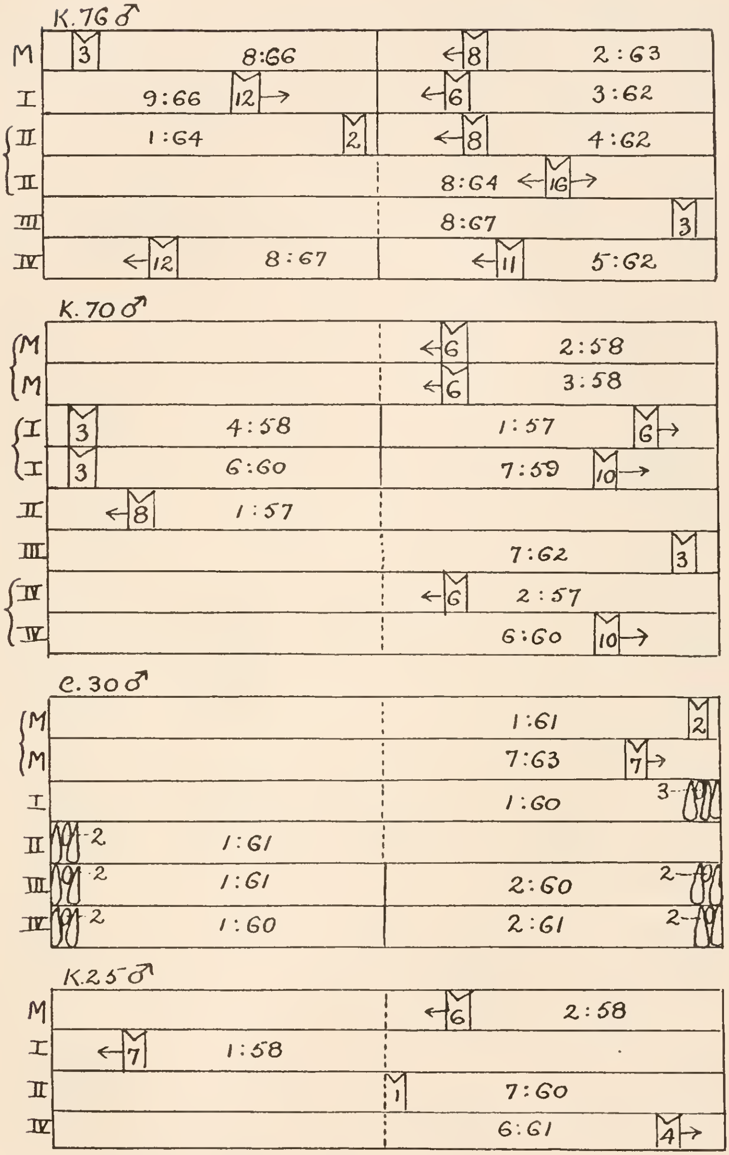

K. 76 ♂
M
I
II
II
III
IV
3
8:66
8
2:63
9:66
12
6
3:62
1:64
2
8
4:62
8:64
16
8:67
3
12
8:67
11
5:62
K. 70 ♂
M
M
I
I
II
III
IV
IV
6
2:58
6
3:58
3
4:58
1:57
6
3
6:60
7:59
10
8
1:57
7:62
3
6
2:57
6:60
10
C. 30 ♂
M
M
I
II
III
IV
1:61
2
7:63
7
1:60
3
2
1:61
2
1:61
2:60
2
2
1:60
2:61
2
K. 25 ♂
M
I
II
IV
6
2:58
7
1:58
1
7:60
6:61
4

FIG. 11.

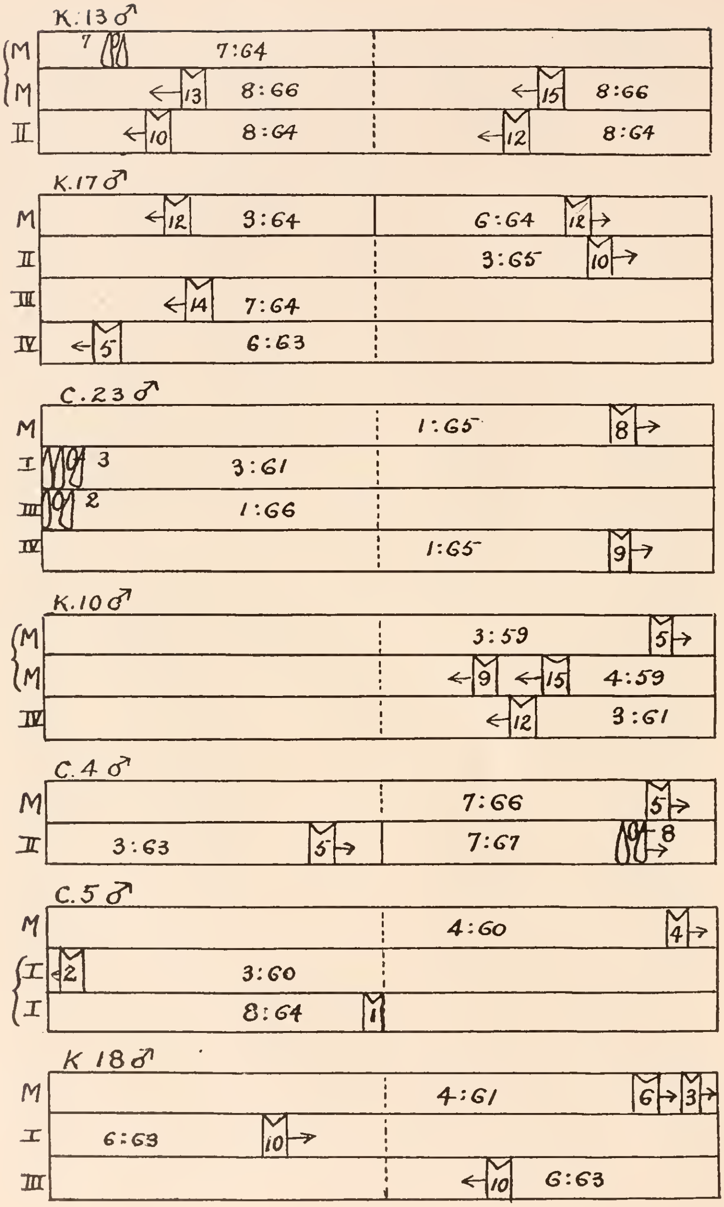

K:13 ♂
M
M
II
7
7:64
13
8:66
15
8:66
10
8:64
12
8:64
K.17 ♂
M
II
III
IV
12
3:64
6:64
12
3:65
10
14
7:64
5
6:63
C.23 ♂
M
I
III
IV
1:65
8
3
3:61
2
1:66
1:65
9
K.10 ♂
M
M
IV
3:59
5
9
15
4:59
12
3:61
C.4 ♂
M
II
7:66
5
3:63
5
7:67
8
C.5 ♂
M
I
I
4:60
4
2
3:60
8:64
1
K 18 ♂
M
I
III
4:61
6
3
6:63
10
10
6:63

FIG. 12.

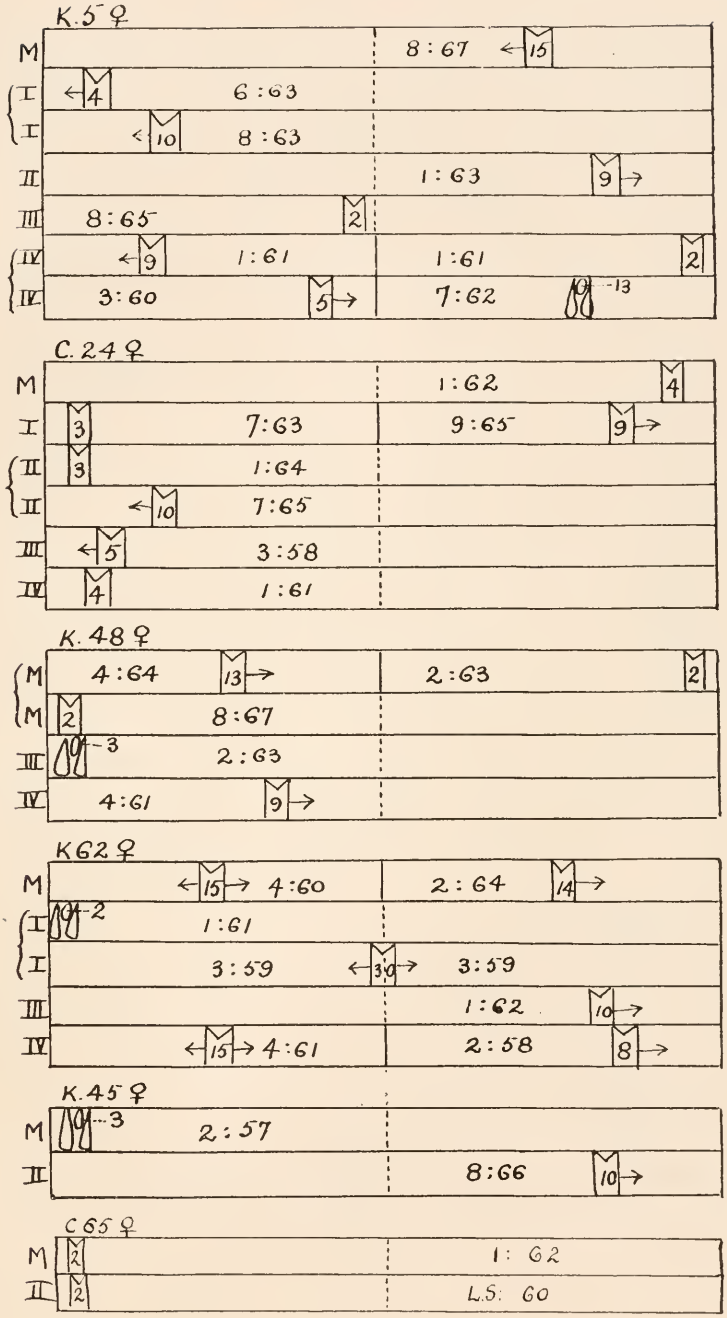

K.5 ♀
M
8:67
15
I
4
6:63
I
10
8:63
II
1:63
9
III
8:65
2
IV
9
1:61
1:61
2
IV
3:60
5
7:62
10
13
C.24 ♀
M
1:62
4
I
3
7:63
9:65
9
II
3
1:64
II
10
7:65
III
5
3:58
IV
4
1:61
K.48 ♀
M
4:64
13
2:63
2
M
2
8:67
III
3
2:63
IV
4:61
9
K62 ♀
M
15
4:60
2:64
14
I
2
1:61
I
3:59
30
3:59
III
1:62
10
IV
15
4:61
2:58
8
K.45 ♀
M
3
2:57
II
8:66
10
C65 ♀
M
2
1: 62
II
2
L.S: 60

FIG. 13.

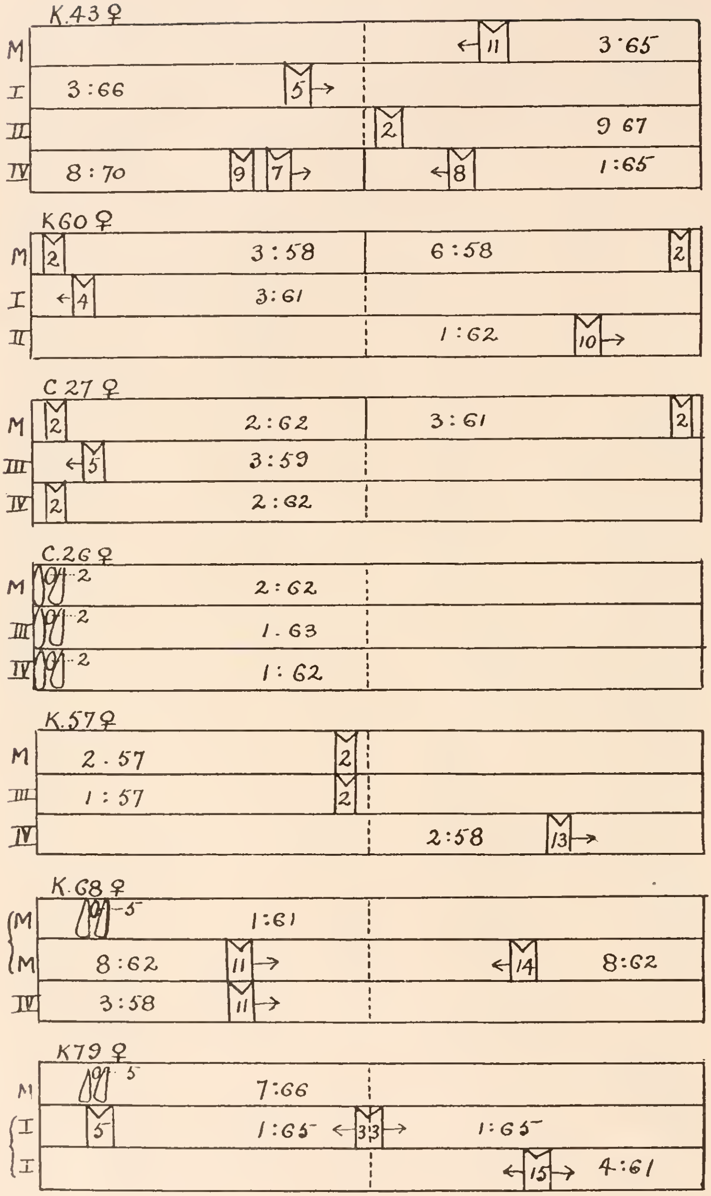

K.43 ♀
M
I
II
IV
3:65
3:66
5
11
2
9 67
8:70
9
7
8
1:65
K60 ♀
M
I
II
2
3:58
6:58
2
4
3:61
1:62
10
C 27 ♀
M
III
IV
2
2:62
3:61
2
5
3:59
2
2:62
C.26 ♀
M
III
IV
2
2:62
2
1.63
2
1:62
K.57 ♀
M
III
IV
2.57
2
1:57
2
2:58
13
K.68 ♀
M
M
IV
5
1:61
8:62
11
14
8:62
3:58
11
K79 ♀
M
I
I
5
7:66
5
1:65
33
1:65
15
4:61

Fig. 14.

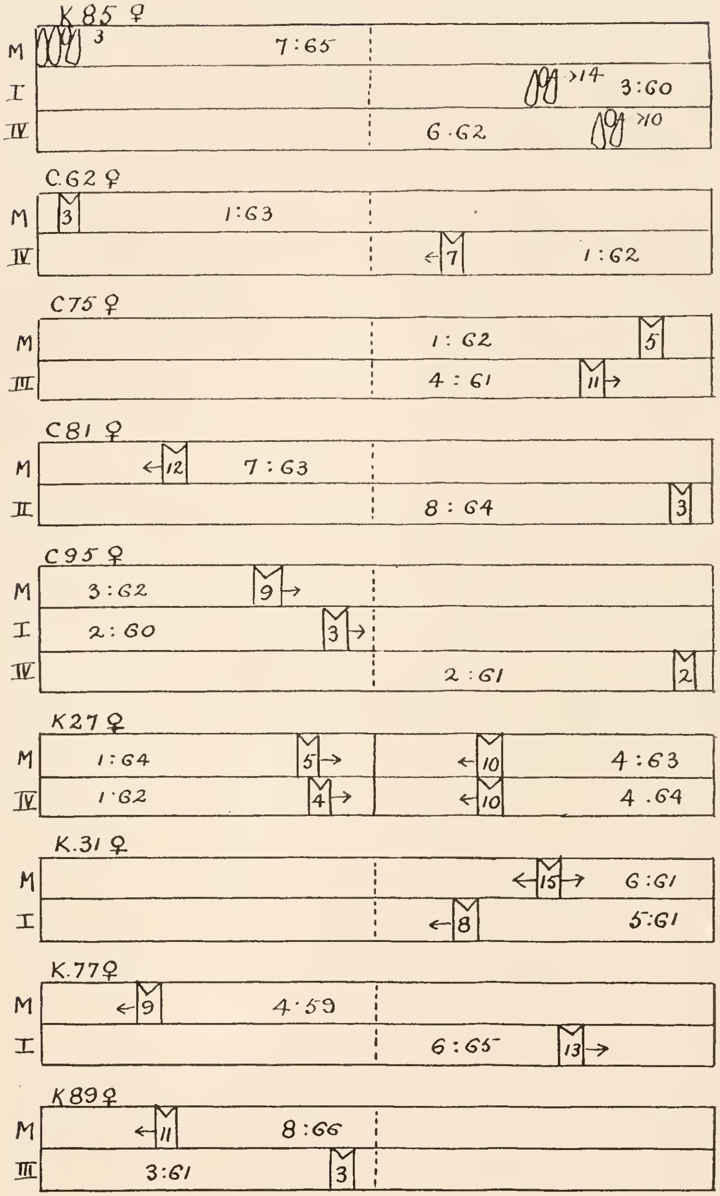

K 85 ♀
M
I
IV
3
7:65
>14
3:60
6·62
>10
C.62 ♀
M
IV
3
1:63
7
1:62
C75 ♀
M
III
1:62
5
4:61
11
C81 ♀
M
II
12
7:63
8:64
3
C95 ♀
M
I
IV
3:62
9
2:60
3
2:61
2
K27 ♀
M
IV
1:64
5
10
4:63
1·62
4
10
4.64
K.31 ♀
M
I
15
6:61
8
5:61
K.77 ♀
M
I
9
4·59
6:65
13
K89 ♀
M
III
11
8:66
3:61
3

FIG. 15.

1. *The Difficulties in the Way of Accurately Analyzing the Data.*

It would be interesting to analyze the data given in the tabulation above, but before an intelligent analysis can be made some of the chief sources of error should be pointed out.

1. Perhaps the most obvious hindrance to accurate analysis of the data appears in the fact that we cannot be certain, if more than one anomaly appears, that the inheritance is solely from the mother, for there must obviously be a fairly large number of sets of quadruplets in which both parents had anomalies. Some sets show clearly this biparental influence, but in many cases it is impossible to decide whether the condition is inherited from the mother alone or is of biparental origin.

2. Another source of error in presenting a pictorial tabulation of the localization of these minute elements is the result of a somewhat arbitrary method of determining the middle of a band, for although I have chosen to locate the middle by dividing the scutes into equal numbers on right and left sides, I feel sure that the arithmetical middle does not always correspond to the morphological middle. There are definite indications in many cases that there are more scutes on one side of the median dorsal line than on the other, in which instances the arithmetical middle is obviously not median. This condition is probably due to the fact that when two parents with decidedly different scute counts mate we may get the maternal number in one half band and the paternal in the other. The middle point therefore is not a very favorable landmark for scute localization but it seems necessary to use it in order to indicate unilateral symmetry reversals. Localization from the lateral margins of the bands is much safer than localization from the middle and it is quite obvious that laterally placed scutes show much closer correspondence in position than do those near the middle.

3. Another factor that shows the inadequacy of the numerical localization method inheres in the fact that different bands vary greatly in number of scutes, ranging from 57 to 70 in the present collection. Now a scute that is pictured as being 5 scutes from the margin in a band of 57 scutes is morphologically farther from the margin than a scute located 5 places from the margin of a band of 70 scutes. So when we are comparing the locality of the

scutes of one individual with another we should bear in mind the numbers of scutes in the various bands dealt with.

2. *Classification of Types of Inheritance and Resemblance among Quadruplets.*

Analysis of the individual cases reveals several well-marked categories:

(*A*) Strikingly close resemblances between mother and offspring, or among the different fetuses of a set.
(*B*) Cases of symmetry reversal between parent and offspring.
(*C*) Cases of mirror-imaging among the fetuses of a set.
(*D*) Cases of half-band reversals of symmetry between mothers and offspring.
(*E*) Cases of half-band reversals between different fetuses of a set.
(*F*) Cases of half-band reversals in the same individual.
(*G*) Longitudinal reduplications down the long axis.
(*H*) Transverse reduplications in a single band.

Each of the categories will herewith receive separate attention.

A. Strikingly Close Resemblances between Mother and Offspring, or Between the Different Fetuses of a Set.—Cases are here dealt with in which the form of the anomaly, its localization and symmetrical relations are the same, or very nearly so, in two or more members of a set, the mother included. The most striking cases are considered first.

Set *K* 27 (p. 189) shows the most remarkable case of exact resemblance. The mother has two double scutes in two different bands, and one of the offspring (fetus 1) has the same double scutes in the same positions of the same two bands as in the mother. Here we have a little difficulty due to locating the morphological middle of the first band. If we take the arithmetical middle, as has been done, the double scute of band 1 of the mother is five scutes from the middle, but that of fetus I. is four scutes from the middle. If we count from the margin, however, the scute is number 28 from the left in both cases, for there are two more scutes to the band in the mother than in the offspring.

Set *C* 26 (p. 188). The mother has a small scute (like Fig. 2*a*) in band 2, two places from the left hand margin. Fetuses II. and IV. (one of each pair) have identical anomalies but shifted to band 1.

Set *K* 57 (p. 188). The mother has scute 2 from the middle of band 2 double, and fetus III. has an identical anomaly in band 1.

Set *C* 30 (p. 185). The mother has a double scute in band 1 situated two places from the right-hand margin. Fetuses III. and IV. have in band 2 an anomalous scute (like Fig. 2*a*) two places from the right-hand margin. These two elements have bilaterally symmetrical equivalents on the left hand side of band 1.

Set *K* 70 (p. 185). The mother has in band 2 a double scute 6 places to the right of the middle. Fetus IV. has an exactly equivalent anomaly.

Set *C* 76 (p. 184). Fetus II. has in band 4 a double scute 12 places to the left of the middle. Fetus IV. has an exactly equivalent element.

B. Cases of Symmetry Reversal between Mother and Offspring.—All cases are to be included here in which an anomaly of the mother is repeated in the offspring but on the opposite half of the carapace.

Set *C* 76 (p. 184). Mother has in band 8 a double scute three places from the *left* margin. Fetus III. has in band 8 a double scute 3 places from the *right* margin.

Set *C* 30 (p. 185). Mother has a double scute 2 places from the *right* margin of band 1. Fetuses II., III. and IV. each have an anomalous element (like Fig. 2*a*) two places from the *left* margin of band 1.

Set *C* 24 (p. 187). Mother has in band 1 a double scute 4 places from the *right* margin. Fetus IV. has in band 1 a similar element 4 places from the *left* margin. Fetus II. has in band 1 a similar element 3 places from the *left*, and fetus III. has in band 3 a similar element 5 places from the *left*. We have here several degrees of exactness in the mirror-imaging of the inherited anomaly.

C. Cases of Mirror-Imaging among the Fetuses of a Set.—Set *K* 70 (p. 185). Fetus I. has a double scute 3 places from the *left* margin of band 6, and fetus III. has a similar element 3 places from the *right* of band 7. Note that these fetuses are not pairs but face each other from opposite sides of the vesicle. Both are secondary bud individuals.

Set *C* 30 (p. 185). Fetuses I. and II., a pair, have striking mirror-image resemblance. Fetuses III. and IV., the other pair, have the same anomalous element bilaterally.

Set *C* 76 (p. 184). Fetus III. has a double scute 12 places to the *right* of the middle of band 2. Fetuses II. and IV. each have identical elements on the *left* side.

Set *K* 18 (p. 186). Fetus I. has a double scute 10 places to the left of the middle of band 6. Fetus III., which faces I. across the vesicle, has a similar element 10 places to the *right* of the middle of band 6.

D. Cases of Half-Band Reversals of Symmetry between Mother and Offspring.—In this group are included cases in which the parent and offspring have the anomaly confined to the same, right or left, half of the carapace, but the symmetry of that half of one is the mirror-image of the same half of the other.

Set *K* 70 (p. 185). Mother has a double scute 6 places to the *right* of the *middle* of band 2. Fetus I. has a double scute 6 places from the *right* hand *margin* of band 1. If this band had been arranged so that the right hand half were simply shifted over to the left side it would have been an ordinary case of mirror-imaging.

Set *K* 25 (p. 185). Mother has a double scute 6 places to the *right* of the *middle* of band 2. Fetus I. has a similar element 7 places from the *left* hand *margin* of band 1. This is a case of a double reversal. Turn either of these half bands end for end and an ordinary mirror-image case would result.

Set *C* 4 (p. 186). Mother has a double scute 5 places from the *right margin* of band 7. Fetus II. has a similar element 5 places to the *left* of the *middle* of band 3, which is evidently the reversed mirror-image of a similar element in band 7 of the same fetus situated 8 places from the *right-hand margin.*

E. Cases of Half-Band Reversals between Different Fetuses of a Set.—These are cases strikingly like the unilateral reversals of finger-print patterns described by Wilder for duplicate human twins.

Set *K* 76 (p. 185). Fetus II. has a double scute 12 places from the *right margin* of band 1. Fetus II. has a similar element 12 places to the *right* of the *middle* of band 1. Again fetus III. has a double scute 8 places to the *right* of *middle* of band 2, and fetus IV. has a similar element 9 places from the *right margin* of band 4. Here each pair of fetuses mirrors the other as to the right half of the carapace.

Set *K* 56 (p. 184). Fetus I. has a double scute 7 places from the *right margin* of band 6. Fetus II. has a similar anomaly 6 places to the right of the middle of band 1 and a second anomaly 9 places from the *left margin* of band 2, and a third anomaly 9 places to the *left* of the *middle* of band 4. Fetus III. has a double element 8 places from the *right margin* of band 7. Fetus IV. has a similar element 4 places from the *left margin* of band 2 and another element 9 places from the right margin of band 8. All of these anomalies are, I believe, directly inherited from the mother, who has a double scute 11 places to the *left of the middle* of band 4, but there are numerous reversals within the set involving a shifting up and down the long axis. This is one of the most complex cases in the collection and would bear careful study as it illustrates most of the symmetry phases seen in armadillo quadruplets.

F. Cases of Half-Band Reversals in the Same Individual.—Set *K* 56 (p. 184). Bands 2 and 4 of fetus II. have a reversed symmetry of the left half.

Set *K* 76 (p. 185). Fetus II. has a reversed symmetry in bands 1 and 4; Fetus IV. has a reversed symmetry in the two halves of band 4.

G. Longitudinal Reduplication Down the Long Axis.—It is fairly common to find an inherited anomaly appearing not merely once but repeated in two or more bands. Sometimes an anomaly of an anterior band is balanced by a similarly located anomaly in a posterior band. The condition is somewhat like that which appears in transverse rows of double scutes that are called double bands, but the tendency for an extended condition of the anomaly is vertical instead of horizontal and is seldom continuous.

Set *K* 32 (p. 184). Fetus II. has a double scute in band 2 and another exactly in a vertical line with it in band 7.

Set *K* 70 (p. 185). Mother has scute 6 to right of middle double in bands 2 and 3. Fetus I. has scute 3 from the left margin double in both bands 4 and 6, also in bands 1 and 7. Fetus IV. has in bands 2 and 6 the same double scutes as fetus I. has in bands 1 and 7, but there is a symmetry reversal in the half bands. There seems to be a strong tendency for the offspring in this set to inherit the vertical reduplication of the mother.

H. Transverse Reduplication in a Single Band.—Here are included cases in which two or more double scutes occur close together but not contiguous in the same band. Such cases are probably in the nature of interrupted band anomalies. Examples may be seen in sets *K* 18, *K* 10, *K* 43, *K* 56. There are as many more cases in sets with normal mothers that are shown in the appendix.

The interpretations offered in the above analysis may be open to objection in certain cases but there can be no controversy as to the nature of the great majority of cases. There is strong evidence of much mirror-imaging between pairs and between individuals of a pair and there are numerous cases of half-band reversals quite like those occasional reversals of finger-print patterns described for duplicate human twins by Wilder, and which Bateson believes to be evidences of a former system of symmetry common to the two individuals before the separation of their primordia. If these rare and vestigial reversals in duplicate human twins are to receive the above interpretation, we should feel no hesitancy about a similar interpretation of the quite frequent occurrence of symmetry reversals in armadillo quadruplets.

C. Discussion and Conclusions.

1. *The Problems of Inheritance and Segregation.*

(*a*) *The Exceptional and Unique Character of the Material.*—No other animal that I know of is so beautifully adapted for the study of specific and individual variability as is the armadillo. The strikingly diagrammatic arrangement of the integument into the five armor shields furnishes an unusual opportunity for accurate enumeration of units and for a study of inter- and intra-individual correlation. These characters, moreover, are definite in numbers and arrangement long before birth. How fortunate a circumstance it is that this species, which is unique in its polyembryonic method of reproduction, should at the same time be unique in its possibilities for biometric treatment! I was especially impressed with this fact lately when I tried to get some basis for a comparison between cattle twins and could find nothing satisfactory for the purpose. The same would be true for sheep, cats or any other mammal. Differences in size, the only measurable differences in these species, are quite unsafe criteria for the determination of coefficients of correlation, since they are notoriously affected by differences in nutrition. Although any part of the integument of the armadillo is superior for the establishment of correlation constants to anything found in any other mammal, the banded region, on account of its regularity, definiteness and specific fixity, is preeminently adapted for the study of inheritance and of the degrees of resemblance and difference among individuals of a polyembryonic set.

So regular is the banding that it would be impossible to discover any symmetry in the bilateral arrangement of integumentary structures were it not for the occurrence of occasional band irregularities (double bands) and of fairly frequent double scutes. These peculiarities of the integument furnish landmarks for the study of symmetry relations that are quite uniquely suitable for such studies and are more readily comparable than are the fingerprint patterns of human duplicate twins that have been used for a similar type of study.

(*b*) *Irregularities in the Inheritance of Band and Scute Anomalies.*—That band and scute anomalies are definitely inherited is proven by the fact that anomalies are always present in offspring of mothers that show the anomaly. The real problem is to explain why all four features of a set of quadruplets do not inherit these anomalies equally and in the same form. Since they come from a single fertilized egg they have the same genetic constitution and should be identical unless there exist certain agencies that result in an irregular distribution of the differentiating factor responsible for anomalies. That these agencies which produce irregular distribution of anomalies among the individuals of a set are not environmental is certain, for there appears to be no environmental inequality except that of nourishment, and differences in nourishment so great as to strikingly affect the size of the individuals do not at all affect the inheritance of anomalies. This could be readily shown by a comparison of sets that show marked size inequality with sets that show size identity.

If then the agency at the basis of irregular distribution of anomaly factors is not environmental, it must be some sort of internal agency. I have been unable to think of any mechanism engaged in either regular or irregular distribution of inheritance factors except the obvious mechanism of mitotic cell division. If this mechanism worked in a perfectly accurate and equitable fashion there would be no opportunity for any irregularities in the distribution of differentiating factors; but there are irregularities and hence the mechanism must lack accuracy.

If the factor or factors for anomalies are present in the oösperm, as we are forced to believe, they must have their seat either in the nucleus or in the cytoplasm. Since the anomalies are evidently as strongly inherited from the father as from the mother, it is hardly likely that the locus of the factors is cytoplasmic, for the male cell has an insignificant cytoplasmic organization. In view of these facts we may safely assume that the locus of the factor is in one or more chromosomes. When all four fetuses inherit an anomaly, we must assume that the factor has been equally distributed to the daughter cells of the first cleavage and probably for several successive cleavages. If, however, the anomaly is confined to a pair of embryos derived from one half of the vesicle, it seems likely that the factor was so distributed as to be totally absent from one of the first two blastomeres. When only one embryo inherits the anomaly the unequal distribution may have occurred during the second or later cleavages.

After a long search for some more satisfactory explanation of the facts than that afforded by a resort to the mechanism involved in cleavage, I have been finally driven back upon this explanation by the facts immediately to be brought out in connection with the phenomenon of somatic and germinal segregation.

(*c*) *Somatic and Germinal Segregation.*—The particular method of polyembryonic reproduction in the armadillo is definitely known to be a process involving a precocious dichotomous budding initiated by the primitive ectoderm. The individual fetuses are produced agamically as bud products and therefore constitute a clone; so whatever somatic variations occur among the four fetuses of a set are obviously types of clonal variation.

Bud or clonal variations have for a long time been propagated vegetatively by cutting, grafts, etc., and are known to hold true to their somatic characters. The explanation of bud or clonal variations that has usually been offered involves the introduction of two causal factors, one internal and the other external. It is said that a local peculiarity may be induced by local conditions outside of the bud tissue itself, such as unfavorable or extra favorable position on the plant; but such an explanation involves many theoretical difficulties. A more acceptable explanation involves the assumption that there is some segregative mechanism that brings about an uneven distribution of inherited factors, so that one type of factor may be present in one part of the growing plant and absent in another. Special cases of somatic segregation are those cases that we have been accustomed to consider under the term *particulate* or *mosaic* inheritance. Such inheritance conditions are supposed to be due to a segregation in a single hybrid offspring of the opposed characters of the two parents. When, for example, a cross is made between a plant with white and one with red petals, we may get a hybrid possessing flowers with some white and some red petals. This is said to be a result of the segregation of parental color factors so that some cells get certain factors and others do not.

So far as I am aware it has not been shown whether in bud or clonal variations, germinal segregation goes hand in hand with somatic segregation. If a green plant that produces a white bud variation should be found to produce flowers on this bud that bred true to the white character, we would have a very clear case of parallel somatic and germinal variation.

Now in the armadillo we have precisely this state of affairs, as can be brought out by a study of the inheritance of double bands and double scutes. For, when some of the fetuses of a set exhibit the anomaly as shown in the mother and others do not, we have unequivocal somatic segregation; but we have something more than this, since those individuals that have the inherited anomaly in the soma also must have the factor for the anomaly in the germ plasm, else how can we account for the fact that whenever a mother has an anomaly it appears in one or more of her offspring?

This segregation must have occurred at a period prior to the separation of primary germ layers, in order to account for the strict parallelism between the germinal and somatic cells, and I have reason to look back to the earliest cleavage divisions as the probable time and occasion of the segregation of determinative factors. This view is arrived at after considering all of the available facts and I believe is justified. Strangely enough we seem once more to be driven back to the cleavage stages for an explanation of the phenomena of twinning, but there is a vast difference between merely assuming the blastotomy origin of twins and accepting the cleavage mechanism as the most probable apparatus responsible for parallel germinal and somatic segregation of inherited factors.

Moreover segregation of inherited anomalies among individuals of polyembryonic sets is one problem, while the symmetric or asymmetric localization of these factors in the soma of the individual is another—the problem of organic symmetry.

2. *The Peculiar Symmetry Relations Among Quadruplets.*

(*a*) *Normal and Reversed Symmetry in the Occurrence of Anomalies and their Significance.*—If no complicating factors entered into the matter of the inheritance of double bands or double scutes, we would expect to find a double scute of the left margin of a given band in the mother inherited as a similarly located double scute by all four offspring. We have attempted to explain the failure of the anomaly to appear in all of the offspring of a litter by positing a segregative mechanism operating during cleavage. Such a mechanism, however, appears inadequate to explain the symmetry relations so peculiar to polyembryonic reproduction.

The mirror-image type of symmetry is normal for the antimeric halves of a single individual and exceptions to or breaches of the mirror-image type of symmetry affords a new problem. Double monsters are also frequently characterized by mirror-image symmetry, due to the fact that the two halves are not physiologically isolated and are therefore parts of a single system of symmetry.

Occasional vestiges of mirror-imaging have been described by Wilder for the finger-print patterns of duplicate human twins, as when the pattern of the right index finger of twin *A* is the mirror-image of the left index finger of twin *B*. These symmetry reversals in duplicate twins have been emphasized as evidences of the monovic origin of such individuals and the reversals themselves have been interpreted as the last trace of an early symmetry formerly common to the two individuals but subsequently largely outgrown.

Now in the armadillo there are many definite evidences of a system of symmetry common to all of the quadruplets, upon which has been superimposed a secondary symmetry system between twins. This in turn is more or less completely obliterated later by a tertiary symmetry between the antimeric halves of the single individuals. In some sets evident traces of the primary system of symmetry persist as mirror-image relations between individuals of opposite pairs, but it is more usual to find no trace of the primary system. The secondary mirror-imaging between pairs is far more commonly in evidence, but is frequently obliterated by the tertiary mirror-imaging between antimeric halves of the same individual, which latter is the prevailing symmetry system. An analysis of this intricate interplay of three grades of symmetry systems is by no means a simple task, but in the foregoing descriptions of individual sets we have indicated our interpretation of the various mirror-image and symmetry reversal phenomena that form the basis of such an analysis. In general, mirror-imaging between individuals of opposite pairs is interpreted as an evidence of the early system of symmetry present in the embryonic vesicle before polyembryonic budding began. When the primary buds are formed they are the product of the antimeric halves of the undivided embryo and therefore should have mirror-image relations, but a partial physiological isolation of the two buds permits a certain degree of reorganization or regulation in the symmetry relations, that tends partially to obliterate the original symmetry relations of the undivided embryo. Similarly, when each primary bud subdivides to form the secondary buds that are the primordia of the definitive individuals, a certain residuum of the primary bud symmetry system is carried over, manifesting itself in mirror-imaging between the twins derived from the same primary bud. But here again a certain amount of regulation occurs so that a third system of symmetry, the bilateral symmetry of each individual, tends to obliterate former systems of symmetry.

A further evidence of the existence of a primary system of symmetry relations is seen in the mode of formation of the secondary buds. Each primary bud gives off to its *left* a secondary bud as though the whole vesicle had a growth whirl to the left. It appears to be acting as a single system at the very time when polyembryonic processes are most active.

(*b*) *The Closer Symmetry of Pairs and its Significance.*—One of the most striking early discoveries resulting from a study of armadillo quadruplets was that the four fetuses of a set are paired so that one pair is fixed to the right-hand placental disc and the other pair to the left-hand placental disc. These discs lie respectively on the right- and left-hand sides of the uterus. The pairs are more strikingly identical than are the quadruplets as a whole and they more often exhibit interindividual mirror-imaging than do the individuals of opposite sides of the vesicle. It at first seemed probable that this condition was due to the "fact," carried over from the literature on the origin of human duplicate twins, that one pair was derived from one and the other from the second blastomere of the two-cell stage. Now that we know more about the embryonic history of the armadillo we are less inclined to relate the symmetry of the vesicle to the symmetry of the oösperm.

It will be recalled that the early blastodermic vesicle, when it becomes fixed to the mucosa of the fundus uteri, adheres by means of a predetermined disc of cells called the "Träger." This disc is evidently derived from the animal pole of the egg and hence we have a mechanism for fixing the principal axis of the embryo and causing it to coincide with that of the mother. The blastodermic vesicle, even if it had a predetermined bilaterality, could hardly fix itself so that its dorso-ventral axis would correspond with that of the uterus, so we are driven to the conclusion that the embryo at the time of fixation has no bilaterality, but acquires its bilaterality from that of the mother. The first indication of bilaterality is seen when the vesicle elongates toward the right and left oviducal openings of the uterus and when bilateral buds of ectoderm grow out to the right and left sides. In other words the bilaterally symmetrical arrangement of the embryonic primordia is imposed upon the vesicle by the form of the uterus.

These first embryonic buds, the primary buds, stand for two pairs of twins, each bud redividing to form two secondary buds, each of the latter going to form a separate embryo. The closer resemblance between the twins of one side is simply the expected result of their community of origin, for they are the product of the longitudinal splitting of a single primary bud of ectoderm. In other words both have been derived from a rather limited area of cells which is separated from the primordia of the opposite pair by a considerable zone of extra-embryonic tissue. On any theory of unequal distribution during cleavage of differentiating factors we would expect these twin products of the horizontal splitting of a common primordium to be closely similar to each other and to show a relatively large amount of mirror-imaging.

(*c*) *Bateson's Views Concerning Twinning.*—In his book "Problems of Genetics" Bateson has an interesting and suggestive chapter on "meristic phenomena" in which he lays great stress upon the fundamental importance of the problem of cell division and other division phenomena. He conceives of the phenomena of bodily symmetry as a direct result of the symmetrical features of cell division. Twinning in mammals is for him a special case of the effects of cell division for he adopts the traditional view that the twins result from a physiological isolation of the blastomeres of the two-cell stage of cleavage. Bateson accepts Wilder's position that duplicate twins are in a series with double monsters of various grades. Double monsters show all degrees of mirror-imaging, while duplicate twins show only vestiges of this phenomenon. Much emphasis is laid upon the few cases of reversed finger-print patterns in the duplicate twins described by Wilder. Any sort of mirror-imaging (symmetry reversal) is an indication of a system of symmetry common to both individuals, whether they are joined or separate.

In closing it may be of interest to state that an examination of a considerable number of sets of quadruplets reveals no indication of a reversal of symmetry in the viscera. As in human duplicate twins symmetry reversals are confined to the integumentary structures. Why should this be so? Bateson propounds this inquiry for duplicate twins as follows: "If anyone could show how it is that neither of a pair has transposition of the viscera the whole mystery of division would, I expect, be greatly illuminated." Now in the armadillo the process of polyembryonic budding that results in twinning is initiated and carried out in the ectoderm, while the endoderm becomes involved only passively and considerably later. Here we probably have the answer to Bateson's inquiry, for symmetry reversals involve only the tissues that are primarily concerned in twinning. How much this statement serves to illuminate the mystery of division I am not prepared to say.

I am inclined to believe that duplicate human twins become physiologically isolated at a considerably earlier period than do armadillo quadruplets, and my reason for this belief is founded on the fact that there is so very little mirror-imaging in the former and so much in the latter. It appears to be a good general rule that the earlier the separation the more complete is the reorganization of symmetry relations in the separated individuals and the less residuum of the original common symmetry. Double monsters doubtless begin to separate comparatively late in ontogeny and hence show very pronounced mirror-imaging. Armadillo quadruplets appear to exhibit a condition intermediate between duplicate twins and double monsters for they have an interesting combination of the effects of an original common system of symmetry and of secondary and tertiary systems of symmetry. Since it seems entirely probable that human duplicate twins are separated at a much earlier period than are armadillo quadruplets, it may not be unreasonable to look for this separation at some period of cleavage. Or there may be a division of the inner cell mass into the primordia of two embryos. The problem of the exact mode of origin of duplicate human twins is however likely to remain unsolved for a long time to come.

Appendix.

Sets of Quadruplets Inheriting Double Scutes from Unknown Fathers.

Purely as a matter of record there is included herewith as an appendix a pictorial tabulation of 31 sets of fetuses from normal mothers but themselves with anomalies. There is every reason to believe that these characters have been inherited from the unknown fathers. There are 15 female sets and 16 male sets. The same plan of representing the occurrence and location of these double elements is followed as was used in the earlier tabulation, the key to which is given on page 181.

Since all of these cases are uniparental while many of the cases discussed earlier are biparental, one would expect a simpler state of affairs and fewer double scutes per set. This is actually the case. The interrelations between the different fetuses of a set are on the average much more clearly defined since there is no confusing admixture of elements from both parents. These facts go far to prove that the conclusions reached in the earlier part of the paper regarding modes of inheritance are well founded.

Many striking cases are seen of close resemblance between twins, of mirror-imaging and half-band reversals between twins, all of which reinforce the conclusions reached on the basis of the data heretofore discussed. Nothing, however, would be gained by calling further attention to individual cases. The interested reader will be able to pick out and classify for himself the various types of anomaly. The pictorial method of recording these elements may need a little study on the part of the reader but is fully justified by the fact that a large amount of valuable data, the like of which may never again be available, is given in very compact form.

Correction.—On page 27 of the first installment of this study (Newman '15) is figured the double band arrangement in set *K* 2. This set was classed erroneously among sets the mother of which had no anomalies. The notebook in which the data was taken shows the mother of *K* 2 was misplaced and later found and has two anomalies. Band 3, which has 64 scutes, has a double scute, like Fig. 2*a*, 16 places to the right of the middle. Band 5 (63

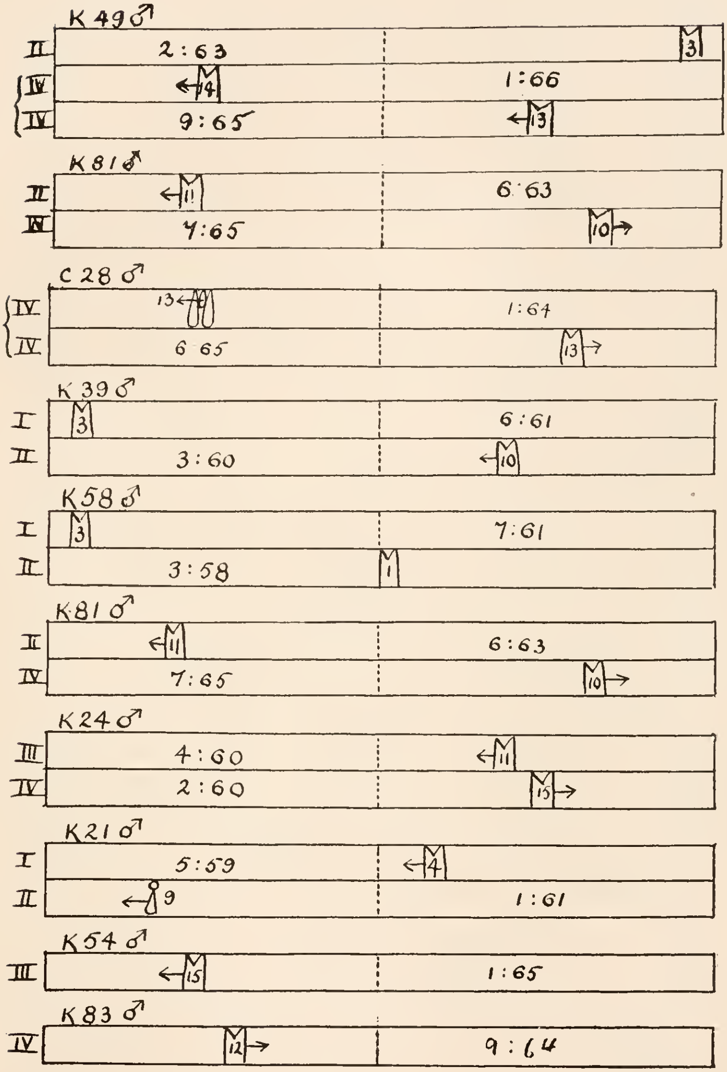

K 49 ♂
II
2 : 63
3
IV
14
1:66
IV
9:65
13
K 81 ♂
II
11
6·63
IV
7:65
10
C 28 ♂
IV
13
1:64
IV
6 65
13
K 39 ♂
I
3
6 : 61
II
3 : 60
10
K 58 ♂
I
3
7:61
II
3 : 58
1
K 81 ♂
II
11
6 : 63
IV
7:65
10
K 24 ♂
III
4 : 60
11
IV
2 : 60
15
K 21 ♂
I
5:59
4
II
9
1 : 61
K 54 ♂
III
15
1 : 65
K 83 ♂
IV
12
9 : 64

FIG. 16.

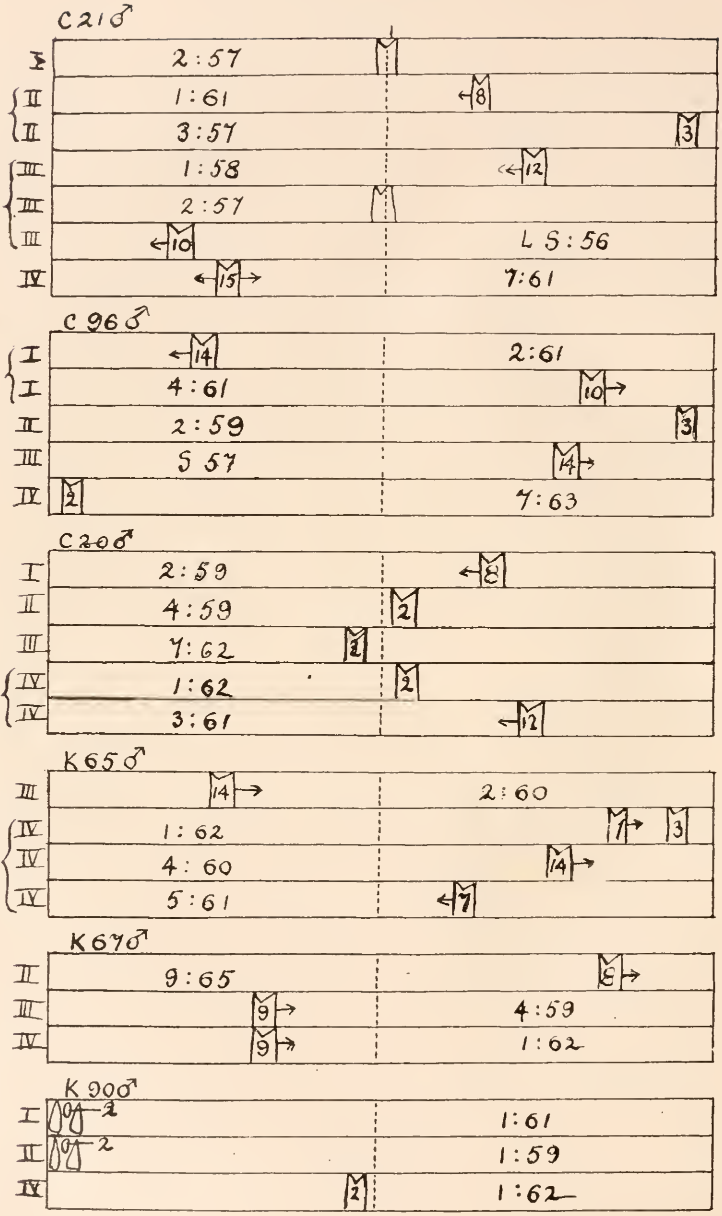

C21♂
I 2:57
II 1:61 8
II 3:57 3
III 1:58 12
III 2:57
III 10 L 5:56
IV 15 7:61
C96♂
I 14 2:61
I 4:61 10
II 2:59 3
III 5 57 14
IV 2 7:63
C20♂
I 2:59 8
II 4:59 2
III 7:62 2
IV 1:62 2
IV 3:61 12
K65♂
III 14 2:60
IV 1:62 7 3
IV 4:60 14
IV 5:61 7
K67♂
II 9:65 8
III 9 4:59
IV 9 1:62
K90♂
I 2 1:61
II 2 1:59
IV 2 1:62

Fig. 17.

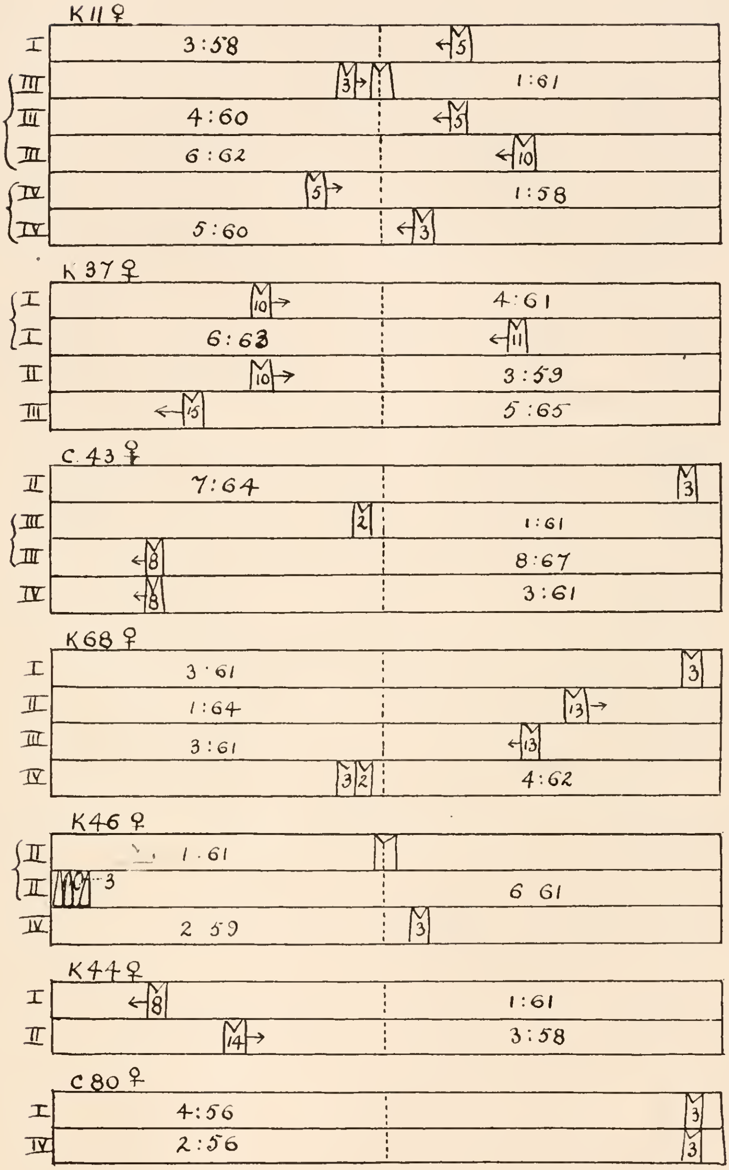

K11 ♀
I
3:58
5
III
3
1:61
III
4:60
5
III
6:62
10
IV
5
1:58
IV
5:60
3
K37 ♀
I
10
4:61
I
6:63
11
II
10
3:59
III
15
5:65
C.43 ♀
II
7:64
3
III
2
1:61
III
8
8:67
IV
8
3:61
K68 ♀
I
3·61
3
II
1:64
13
III
3:61
13
IV
3
2
4:62
K46 ♀
II
1·61
II
3
6 61
IV
2 59
3
K44 ♀
I
8
1:61
II
14
3:58
C80 ♀
I
4:56
3
IV
2:56
3

FIG. 18.

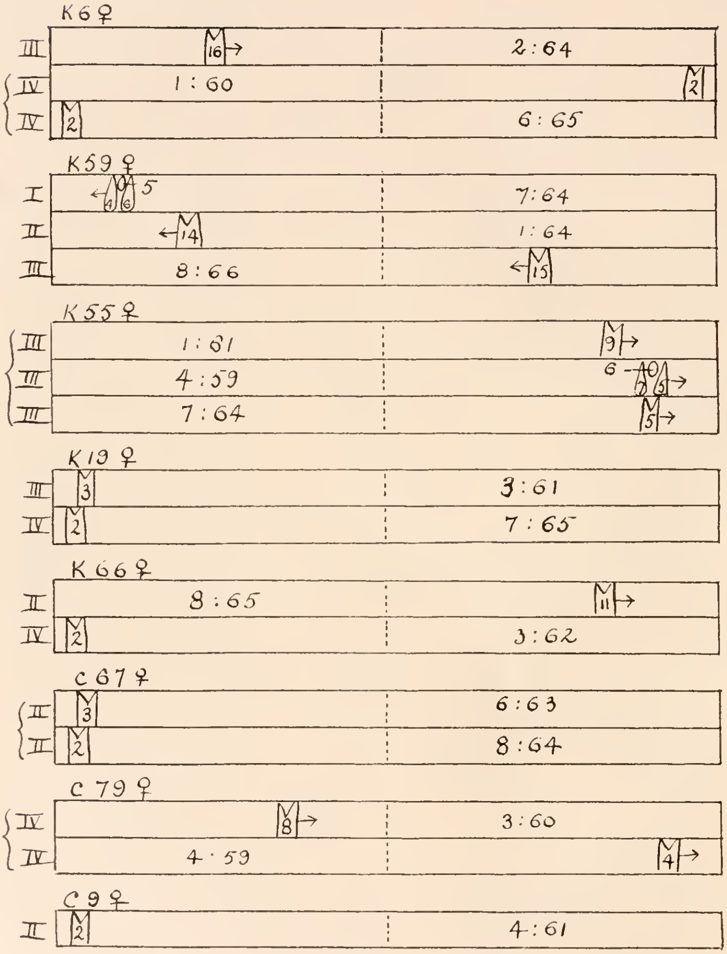

K6♀
III
M 16
2:64
IV
1:60
M 2
IV
M 2
6:65
K59♀
I
4
5
6
7:64
II
M 14
1:64
III
8:66
M 15
K55♀
III
1:61
M 9
III
4:59
6
10
7
5
III
7:64
M 5
K19♀
III
M 3
3:61
IV
M 2
7:65
K66♀
II
8:65
M 11
IV
M 2
3:62
C67♀
II
M 3
6:63
II
M 2
8:64
C79♀
IV
M 8
3:60
IV
4:59
M 4
C9♀
II
M 2
4:61

FIG. 19.

scutes) has a double scute 5 places from the right-hand margin. These two anomalies mark about the inner and outer limits of the band doublings seen in the offspring. This set shows almost perfect mirror-imaging of the whole set of quadruplets. The two primary bud fetuses II. and IV. mirror each other since both have the anomaly bilaterally; the two secondary fetuses I. and III. are also mirror-images, III. having the anomaly on the right and I. on the left. All of these anomalies occur in band 1.

BIBLIOGRAPHY.

Bateson, W.

'13 Problems of Genetics. Yale Univ. Press.

Newman, H. H. and Patterson, J. T.

'09 A Case of Normal Identical Quadruplets in the Nine-banded Armadillo and its Bearings on the Problems of Identical Twins and of Sex Determination. Biol. Bull., Vol. 17, No. 3.

'10 The Development of the Nine-banded Armadillo from the Primitive Streak Stage to Birth; with Especial Reference to the Question of Specific Polyembryony. Journal Morphology, Vol. 21, No. 3.

'11 The Limits of Hereditary Control in Armadillo Quadruplets: A Study of Blastogenic Variation. Journal Morphology, Vol. 22, No. 4.

Newman, H. H.

'13a The Natural History of the Nine-banded Armadillo of Texas. American Nat., Vol. 47, Sept., 1913.

'13b The Modes of Inheritance of Aggregates of Meristic (Integral) Variates in the Polyembryonic Offspring of the Nine-banded Armadillo. Journal Exper. Zool., Vol. 15, No. 2.

'15 Heredity and Organic Symmetry in Armadillo Quadruplets. I. Modes of Inheritance of Band Anomalies.

Patterson, J. T.

'13 Polyembryonic Development in *Tatusia novemcincta*. Journal Morphology, Vol. 24.

Wilder, H. H.

'04 Duplicate Twins and Double Monsters. Amer. Jour. Anat., Vol. 3.

Vol. XXX. *March, 1916.* *No. 3*

BIOLOGICAL BULLETIN

PALM AND SOLE STUDIES.

HARRIS HAWTHORNE WILDER.

(*Continued from page 172.*)

V. Friction-Skin Correspondence in Pygopagi.

A long series of studies of the palm and sole markings in human twins, both *duplicate and fraternal*,[1] has established two principles for twins of the true duplicate type (monochorial, monosexual, striking facial resemblance, etc.), viz: (1) that the corresponding palms and soles in the two individuals are strikingly alike in friction-ridge configuration, even duplicating singular and unusual features, and (2) that the correspondence in the two sides of the same individual is far greater than is usually the case, so that the four hands involved, or the four feet, present so many copies of practically one picture. To these may be added a third condition, not absolutely constant, but frequently noted; a reversal of the pattern of the index figures in the two individuals, affecting either the two right hands or the two left hands, or occasionally both sets.

In this connection it is a matter of great importance to ascertain the condition of the friction ridges in "conjoined" twins, that is, in twins which have never completely separated, but remain throughout life united by certain common parts (i. e., double monsters). The numerous specimens of this sort found in teratological museums have yielded some results, which seem to accord with the findings in separate duplicate twins, but such objects are unsatisfactory to study since they are mostly embryonic, or newborn, and the ridges in consequence are very soft and indistinct, while the fluid in which they are preserved renders a print difficult or impossible.

Recently, however, I have had the opportunity of making careful observations of a pair of living conjoined twins, who were born in May, 1912, and are consequently in their fourth year at the present writing. These twins, which I studied the first time a day or two after birth, are typical pygopagi, of the well-known but rare type represented by Rosa–Josepha Blazek, and Millie–Christine;[1] are united in the sacro-iliac region, but placed somewhat obliquely, so that, instead of looking in directly opposite directions, they are rotated about 45° towards the same side.

---

[1] Wilder, 1902, 1904, 1911.

It thus happens that, while lying or sitting they present to the observer what would be called a front and a back side, considering the entire double monster as a unit, so that when the observer is stationed upon the "front" side the two faces are turned 45°

---

[1] Double twins of the pygopagous type are extremely rare. Baudouin, in 1902, was able to cite but eight authentic cases that have lived, known to the medical profession, and these span an interval of eight hundred years. These are as follows:

1. The "Biddenden Maids"; Biddenden, Co. Kent, England, b. 1100. d. 1134.
2. Case cited by Lycosthenes, writing in 1665. These were girls, b. in Verona, Italy, in 1475.
3. The Hungarian Sisters, Helena–Judith; b. near Komorn, Hungary, 1701, d. 1723.
4. Case cited by Wolff; observed in Russia in 1778, lived but two months.
5. Millie–Christine, negresses; b. in South Carolina, exhibited at Edinburgh in 1856, and later on in the United States. [They died about 1912.]
6. Case of Joly and Peyrat, Jeanne–Marguerite Bombail; b. 1874, at Mazéres, Dept. Ariége, France. Died 24 hours after birth.
7. Case of Pilat, 1879. Born prematurely and one died during delivery.
8. The Bohemian Sisters, Rosa–Josepha Blazek; b. Jan. 20, 1878, and still living (1915). One of these is reported to have borne a normal child. These sisters are well educated, speak several languages with fluency, and have been extensively exhibited.

To this list must now be added (9) the "Samar Twins" Lucio–Simplicio, two boys from the island of Samar, in the Philippines, also (10) the subjects of this paper. The boys are about six years of age at this writing, the girls three and a half. Both sets were born since the above compilation was made.

That similar cases have occurred among primitive peoples, and in the past, is evidenced by the occurrence of folk-lore tales, describing such double twins with some degree of care, and rendering it probable that they were pygopagi. Thus there are the two sisters in an old Samoan tale already quoted by me in my paper on "Duplicate Twins, etc."; there is also a Sioux legend of a double woman, cited by Dorsey in *Ann. Rep. Bureau Eth.*, Vol. 11, 1889–1890, p. 480, with a native drawing.

This exhausts the list, so far as known to the writer, excepting fetuses in museum collections. Any additions to this list would be gladly received. It has been frequently observed, by the writer and others, that double twins, and probably separate duplicates, are female in the great majority of cases. This seems well borne out by the above list.

away from the middle line upon either side, so that the two median bilateral planes form an angle of 90° with each other. In accordance with the method employed in former papers, the individual which is on the left hand of the observer, when he confronts them, is designated as *A*; the other, upon his right hand, *B*. In this case *Mary* is *A*, and *Margaret* is *B*.

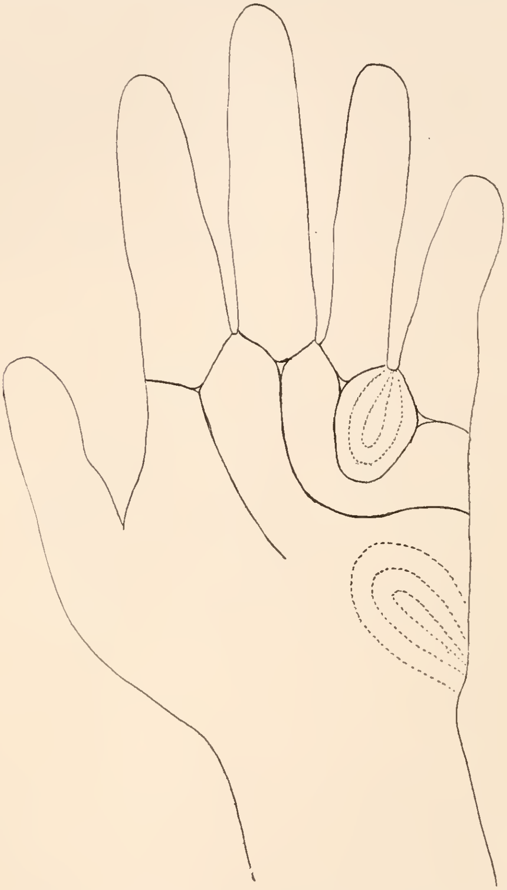


FIG. 26. General pattern for all four hands of the pygopagous twins G——— This was sketched from the rather unsatisfactory studies made upon the active hands of infants of two years. All four hands were of this type.

I studied the palms and soles of these little girls in May, 1914, within a few days of their second birthday. They were large and well-developed children, but had not yet learned to walk, since in attempting it, they would naturally start in nearly opposite directions. They were sitting in a double high chair with a platform in front of them, and appeared almost like two ordinary children, sitting very near together.

I succeeded in taking fairly good prints of their soles, by inking the feet themselves, and then applying quickly a paper pad held flat in the hand, and in this manipulation I was aided by the platform in front of them, which prevented their seeing just what was going on. Prints of the palms, however, in the case of these lively and psychologically alert infants, did not seem feasible, as their hands and fingers were in continual action, and as they were not old enough to have the matter explained to them. The hands were therefore studied as we best could, by the aid of a lens, while the children were being otherwise entertained, and certain of the more conspicuous details were made out. If the children live, as is altogether likely, we may hope for actual prints in three or four years.

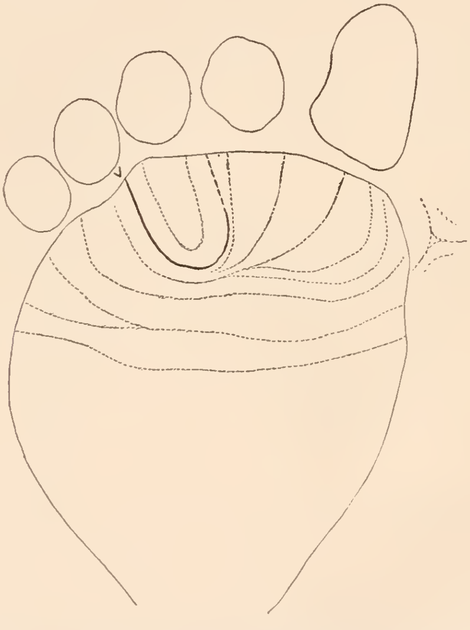


FIG. 27. Print of left sole of Margaret (Component *A*).

Concerning the palms it was fairly definitely made out that *all four are practically alike, and that they conform in both particulars given above to the condition found normal in twins of the duplicate type.* In each of the four there is a conspicuous hypothenar loop opening to the ulnar margin, and in each there is also a large loop covering the fourth interdigital area, and opening up between the ring- and little-fingers. Judging from these data the hand pattern for all four hands cannot be very different from the sketch here submitted, with lines *C* and *D* confluent, and with line *B* reaching the margin above (distal to) the hypothenar pattern. (Fig. 26.) Line *A* must be left uncertain, as it can perfectly well

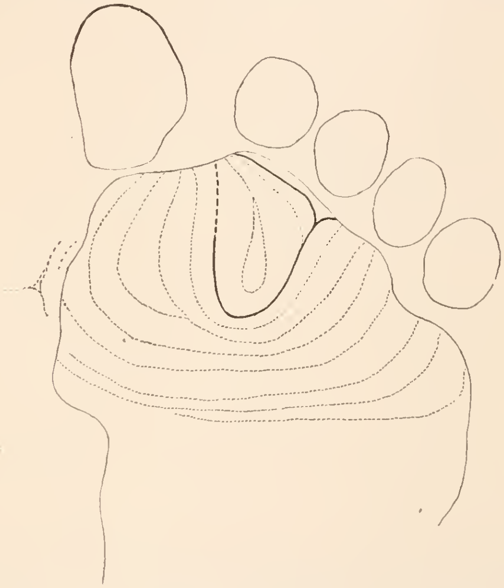


FIG. 28. Print of right sole of Margaret (Component *A*).

follow parallel to *B*, enter the pattern and become involved in it, or emerge below it, that is, assume positions 5, 4, or 3. This will give the formula 8·6·5·5, with a possible variation in the last term. The correctness of this surmise can be readily tested as soon as the little girls are old enough to assist in the printing.

The prints of the feet, taken as they were, were necessarily poor, but enlarged photographs of the results obtained brought out all the essential details, and it is from these that the outlines here given were taken. As the photographs were taken by a scientific photographer, and as distortion was avoided, these outlines may be absolutely trusted. The only improvements that better prints could show would be in the details of individual ridges, and other matters which, even in duplicate twins, are individual, and without significance here.

From a comparison of these four outlines (Figs. 27–30) it will be seen at once that here is presented the unexpected; that while three of the four feet, as well as all four hands (presumably) conform closely to the requirements for typical duplicates, and that in three of the feet even the curious reduced hallucal pattern, an extremely rare type, is faithfully reproduced, yet in the fourth, the right sole of Mary, there is a radical departure from this type, and a loop of the "A" type is presented instead, *i. e.*, a loop opening up between the first and second toes. Furthermore, there is also in the same print, but not in the others, a well-developed fourth interdigital pattern, lying between the fourth and fifth toes; the presence of two lower triradii, quite absent in the other three soles, are also to be detected.

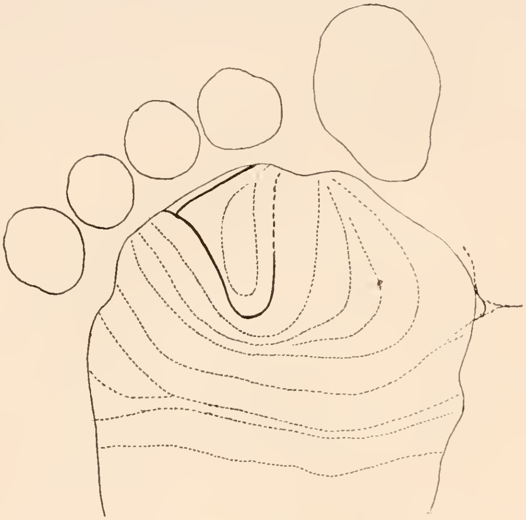


FIG. 29. Print of left sole of Mary (Component *B*).

Taking up these two differences in order, the hallucal pattern of the three feet which are alike is of the extremely rare type *AC*, in which the two triradii *a* and *c* of the typical whorl are absent, and the ridges consequently escape in both directions through the points naturally held by *a* and *c*, that is, distally between the great toe and the second, and proximo-laterally towards the region of the fourth interdigital area and the outer edge. As shown in the actual prints, or in the tracings from them, here presented, the *b* triradius, which is necessary to the full understanding of the relationship, is not shown, but there is no question but what it is present, beyond the tread-area, as indicated by the dotted triradius drawn into each figure.

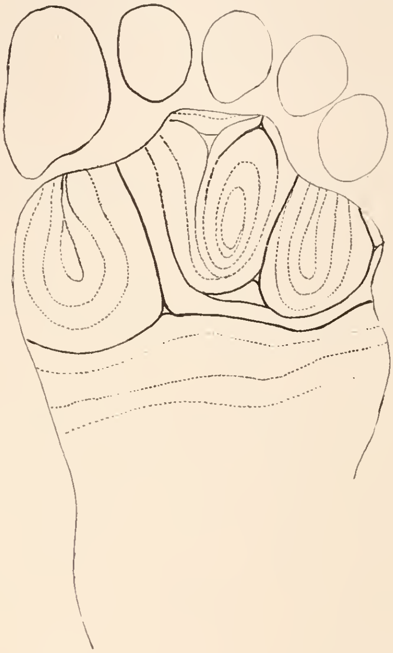


FIG. 30. Print of right sole of Mary (Component *B*).

The difference shown in the aberrant hallucal pattern of Mary's right foot consists in the presence of the *c* triradius, thus closing up all escape for the ridges in that direction, so that consequently they curve around the core and open with the others between the great toe and the next. The essential aberrancy of this pattern consists thus in the presence of a triradius absent in the other cases.

The other difference in this aberrant foot, that affecting the third interdigital pattern, is also due to the presence of a "lower triradius" between the third and fourth interdigital areas, thus rounding the proximal ridges of both into loops. The two proximal triradii shown in the print may be on the other feet as well, placed beyond the limit of the actual print, and of this explanation for the outer one, *D*, we can be certain, as it is never wanting, but occasionally beyond the limit of the tread-area. The other, closing the third area distally, looks like a departure from the other three soles, thus making the total aberrancy of this foot due to the presence of three separate triradii, which are wanting in the other three.

Summarizing the results of this investigation we have, in all four hands, and in three of the feet, the typical correspondences found only in true duplicates, also the right and left correspondence usual in such cases. That these two individuals are really duplicates there is no possibility of doubt since, in addition to the close facial resemblance, which is typical, and the sex, which is female, the commoner sex for duplicates, they were monochorial and had a double, bilobed placenta. As I was fortunate enough to be on the spot at the time of the birth, I secured the afterbirth, which had been preserved with great care by the attending physicians. There was a single chorion, without trace of a separating partition, and the placenta was bilobed, and nearly as large as two normal placentæ. The umbilical cord was a single one for 11 cm. from the placenta, and proceeded from the margin, at the point of bilobing, that is, at the notch between the two halves.

This common cord contained the usual two arteries and a single median vein, and at the forking of the cord to supply the two umbilici the vein split into two branches, so that one of the arteries and one branch of the vein continued into each individual cord. The cord supplying Margaret, now and always the heavier infant, was 11 cm. long from the fork to the ligature; that supplying Mary was 6. Margaret's cord was also somewhat greater in caliber. Assuming that the ligatures were made in both cases at about the same point these latter figures have a meaning, otherwise they are of little value.

We have thus a conjoined pair of true duplicate twins, monochorial, monosexual, and with striking facial similarity. The four hands and three of the feet correspond as closely, as has been found in other cases of separate duplicates; but one of the feet shows a series of departures from the type, all due to the presence, or retention, of triradii lost in the other cases. Once or twice in my other sets of apparently duplicate twins I have found about the same degree of difference in one of the members, which has caused me some doubts, and has weakened the argument in the judgment of others, notably Newman and Patterson, 1911, pp. 856–860.

In this case, however, we have twins who are undoubtedly duplicates, and in which the variation is marked in one member of the eight involved, the others showing the typical correspondence in comparing both the two individuals, and the two sides of the same individual. The case is thus of great service in showing the limit of hereditary control affecting a case where the individual differences are expressed as varying degrees of degeneracy or loss of the features of a complex ancestral configuration.

After the presentation of this new piece of evidence on the subject of palm and sole correspondences in duplicate twins, it would seem a fitting time to review briefly the question of the origin of such cases, especially since a flood of light has been shed upon the whole subject by the investigations of Newman and Patterson on the armadillo.

The early stages of normal human individuals during the time in which we are to expect to find the initial stages of the doubling process are practically unknown, and it would be an almost inconceivable chance which would furnish the material for the study of these stages in human eggs destined to produce twins. We have long been wont, however, to rely in such cases upon the analogies furnished by related mammals, and to fill in the breaks in our knowledge by investigation of material taken from other forms. In this way the normal embryology of single human embryos has been well made out, and in a way that is generally accepted, although actual human material, covering important periods of development, is thus far wanting.

In the armadillo the egg is normally polyembryonic, always producing monosexual individuals which, by the number and arrangement of their scales, prove themselves to be duplicates.

In this animal the early stages are now quite fully known, excepting cleavage, and the first visible signs of multiple individuals are here seen in a process of budding of the embryonal anlage. Almost spontaneously comes the impulse to attribute the origin of human twins and other multiple births to a like process, as was done by Patterson at the time of his discovery; writing: "I am therefore bold enough to suggest that the conclusions which I have drawn concerning the origin of the embryos in this mammal may also apply to cases of duplicate twins and double monsters in the human species." To render this still more plausible he then refers to certain similarities in the early embryology of the two animals considered, man and the armadillo, particularly as shown in the Bryce and Teacher embryo, 1908, a similarity which renders this process in man mechanically possible.

Although this conclusion may seem at first to place the origin of these multiple individuals at the time the budding process is first manifest, there is still much evidence to show that this process rests upon an earlier process which conditions it, that is, that *while the visible process of budding first reveals the multiplicity of individuals developing from the egg, the true cause is to be sought further back*, or, as Newman and Patterson, as well as myself, stated at the beginning of our investigation, in some sort of divison (or we may now say, differentiation) of the early blastomeres.

It is now clearly evident that all ideas of an actual physical separation of these blastomeres, a definite *blastotomy*, does not take place, yet many things still point to the conclusion that a similar condition is obtained through some form of differentiation, and that each of the separate embryonal anlages, be they two or four or eight or eleven (a possible number in *Tatusia hybrida*), is the lineal descendant of a single blastomere, formed during early cleavage. This was the view of Newman and Patterson in 1910, who then wrote: "It seems highly probable that the tissues involved in each of the four quadrants of an embryonic vesicle do really arise as the lineal descendants of one of the first four blastomeres." They further believed, as they definitely expressed farther on, that this differentiation was not a blastotomy in the sense of anything involving a separation, and supposed that even if an embryo were found in the 4-cell stage, it would present to the eye no differences from that of any other mammalian embryo of four cells. The exact truth of this supposition can be known only by a thorough knowledge of the cell-lineage, from the fertilized egg up to the beginning of the budding process, and although such a task is well-nigh inconceivable with our present technique, the results already obtained render even this possible in the future, perhaps by a culture of eggs outside of the body of the mother.

In 1913, after his important studies of the early embryology of the armadillo, Patterson seems to diverge a little from this view of so early a differentiation, and seems almost to confuse this with blastotomy, or at least to feel that blastotomy is necessarily involved in the process, for he says: "The evidence that has been presented in the first part of this paper [describing the budding process] makes it certain that polyembryonic development in the armadillo cannot be explained on the basis of a spontaneous blastotomy, *in the sense that each embryo is the lineal descendant of a single blastomere of the four-celled stage*, and it causes me to view with some doubt the conclusions of this same nature that have been drawn by those who have worked on other polyembryonic forms." The italics are my own, as I wish to call attention to what seems to be his interpretation of blastotomy, whereas the two things, (1) a separation of the blastomeres, and (2) a line of cell lineage which causes the separate anlages to develop each as the descendant of one of them, are not at all the same, and have no necessary connection. The passage is a little obscure, and the author may not mean to express my interpretation, but it certainly seems as though he has abandoned any connection between the numerous embryos and the first four cells, and considers that the separate individuality commences with the budding process very much later. Whatever may be this author's opinion on this subject, which is very likely not as inferred from this sentence, it would seem to me impossible to get such a close correspondence as has been shown in the armadillo, and in human twins, without starting the separation of identities before there has been any appreciable differentiation in the material that controls the development and eventual arrangement of the parts in question, and I am of the opinion that a carefully established cell lineage would substantiate the claims of the two authors in 1910, quoted above, or their statement of 1909, "In the case of *Dasypus* [*Tatusia*] each embryo probably arises from one of the blastomeres of the four-celled stage," which does not open the question of whether these blastomeres were actually separated from one another or were only the ancestors of the separate embryonal anlages of a later time. Concerning the blastotomy theory, that is, an actual separation of the blastomeres, I may take this occasion to state that I no longer believe it, since, among other reasons, comes the very cogent one that such a separation in man, or in any mammal, would necessarily produce as many separate and distinct choria, each with its own attachment to the uterus, a condition which is found in fraternal births, each from a separate egg, but never in true polyembryony. In turning to my earlier work, however, I find this theory stated in unequivocal phrase, as "the total separation of the first two blastomeres of a single egg" (1904, p. 462) and I take this opportunity to recant so far as the "total separation" is concerned, while still adhering to the first two blastomeres as the probable ancestors of the two later embryonal buds respectively.

While engaged in noting changes of opinion, I may say further that the view of a different origin for double monsters with unequal components, autosite and parasite, which I asserted in my paper of 1904, pp. 462–463, I rejected utterly later on, 1908, p. 362, and considered such cases as in origin exactly like other double monsters (including separate twins), and that the deformed condition had been brought about by some accident which deprived one of the components of either its normal amount of nourishment, or prevented a normal growth in some mechanical way. As my first view only has been quoted by Newman and Patterson this seems to be an instance of the very common case in which an error is published much more widely than its later correction. Such modifications, or flat rejections, of former views are naturally incident to all progressive thinking, and in a subject so complex and so vital as the present investigation are inevitable.

Concerning the causes which produce a double monster, that is, twins which have some postembryonal parts in common, and are consequently joined together, the principle of budding, as seen in the armadillo, furnishes some clues as to the possible mechanism of the process. Even in normal twins certain of the embryonal parts are held in common, as always in the armadillo, but as these common parts are embryonic (trophoblast) and are abandoned at birth, they are not apparent later. In double monsters the common parts include more than trophoblastic material and the components are consequently not liberated by the cutting of the umbilical cord.

In a morphological sense the bands of the armadillo carapace, with their variations, and the friction ridges of human palms and soles are partially or wholly homologous structures, so that their use in determining the degree of similarity of twinned individuals is equally warrantable in both cases, while the results may well be compared. Both deal with epidermic structures, the probable homologs of reptilian scales, placed in rows; in both are observed the similar phenomena of the forking and consequent doubling of the lines thus formed. Even the double scale of the armadillo may have its counterpart in a twin sweat-pore, which indicates the composite nature of the unit to which they belong.

While, however, the study of the armadillo has the great and undeniable advantage of allowing the study of the actual embryonic conditions in each case, there are also certain marked advantages in the study of the human palm and sole configuration as a similar criterion of similarity of individuals, and in following the action of heredity. In the first place there is (1) the far greater complexity of pattern, giving an almost infinite number of points for comparison. Here the fineness of the lines, and their occasional participation in the formation of a complex pattern, allows in number and arrangement, to say nothing of the *minutiæ*, that is, the composition of the individual ridges, a complexity far surpassing anything met with in the carapace of the armadillo, where the details concern only nine rows of scales, with some 50–60 unit scales in a row. Another very important advantage is seen in (2) the fact that the records of human twins may easily be made complete, with prints of both parents, father as well as mother, and sometimes a second preceding generation, together with collateral lines, as represented by brothers and sisters, aunts and uncles. In the work on the armadillo the mothers only are known, and thus all peculiarities of the offspring not accounted for by the maternal carapace, are, rightly or wrongly, attributed to the unknown father. As a third advantage, (3) largely one of convenience, may be mentioned the ease with which even a very large collection of flat prints may be filed and handled. This is especially noticed when a series of prints are to be compared at one time.

On the other hand, there are numerous marked advantages accruing from the study of the armadillo, so many, in fact, that the work is indispensible in the study of human friction ridges. The way in which the results obtained in this field have supplemented the work on human twins, rendering vaguely expressed ideas definite, and raising hypotheses to the category of what by analogy may be considered facts, has been the subject of the preceding pages.

In their paper on the limits of hereditary control in the armadillo (1911) the two authors often quoted offer two objections to my conclusions of a similar nature, as shown in the friction ridge configuration of human twins. The first, and most valid one, is that "the origin of the two individuals from a single fertilized egg is assumed from the facts of resemblance," while the second is that "the comparison between the twins is made only after years of post-natal life." Owing to the abundantly proven unchangeable nature of human friction ridge configuration the second objection is invalid, so that there is left but the first one which may become expanded into two questions, that the early conditions in the embryonic history of human twins is unknown, and that in individual cases it is not usually possible to find out whether they were monochorial or bichorial.

As explained above, analogy with the very similar early stages in the armadillo shows pretty conclusively that human monochorial twins are due to a genuine polyembryony, while in some cases it *is* possible to obtain the chorionic relations, as in the case of the double monster above treated. After collecting the details of a twin case from any responsible physician the case need not be watched longer than three or four years to get a set of satisfactory prints. These objections have, however, some validity as applied to my definite claims at the time when I first made them (1902, 1904, 1908), and I am only glad that my hypotheses of that time have been so ably and satisfactorily substantiated by the results of the investigation of the armadillo.

That the formation of multiple embryos in man and the armadillo is due in both cases to a similar cause seems to me undoubted, and was similarly considered by Patterson, who writes (1913, p. 560): "There has been considerable speculation as to how 'indentical twins' and similar types of development have arisen, and I believe that these studies on the development of the armadillo will at least indicate how these phenomena may have come about." He further considers it probable that "specific polyembryony in the Dasypodidæ began by the formation of a set of twins, perhaps at first a sporadic case of gemelliparous development such as probably occurs in the production of duplicate twins in the human species." It is needless to state that I, too, think the same, and because of this belief can go farther and state that whatever is learned in the future concerning the actual causes of polyembryony in the armadillo will solve also the same question concerning the causation of human duplicate twins and double monsters.

To close this subject with a pure speculation, suggested by the work upon the armadillo, Newman and Patterson find (1915) that the quadruplets may be arranged in pairs, I+II, and III +IV, and that the identity of scale configuration is much greater in the two members of a single pair than in members of different pairs. Is it conceivable that human duplicates may bear to each other these two degrees of similarity found here, and that the few cases, like my Nos. VII and XIII, where the palm and sole similarity was not so great as was to have been expected from the facial resemblance and correspondence in sex, may be explained by a relationship such as would be found in the armadillo by comparing II with III, or I with IV? This might involve the early abortion of additional embryos, or might deal with the question of the two *vs.* the four-celled stage, as suggested by Newman and Patterson (1909, p. 186) that "the two in-

individuals derived from one of the blastomeres of the two-cell stage ought to be more nearly similar to each other than to the individuals of the other blastomere." Further investigation in the early embryology of the armadillo, particularly the cleavage stages and the cell lineage, are bound to assist in clearing up these problems, and will be awaited with interest.

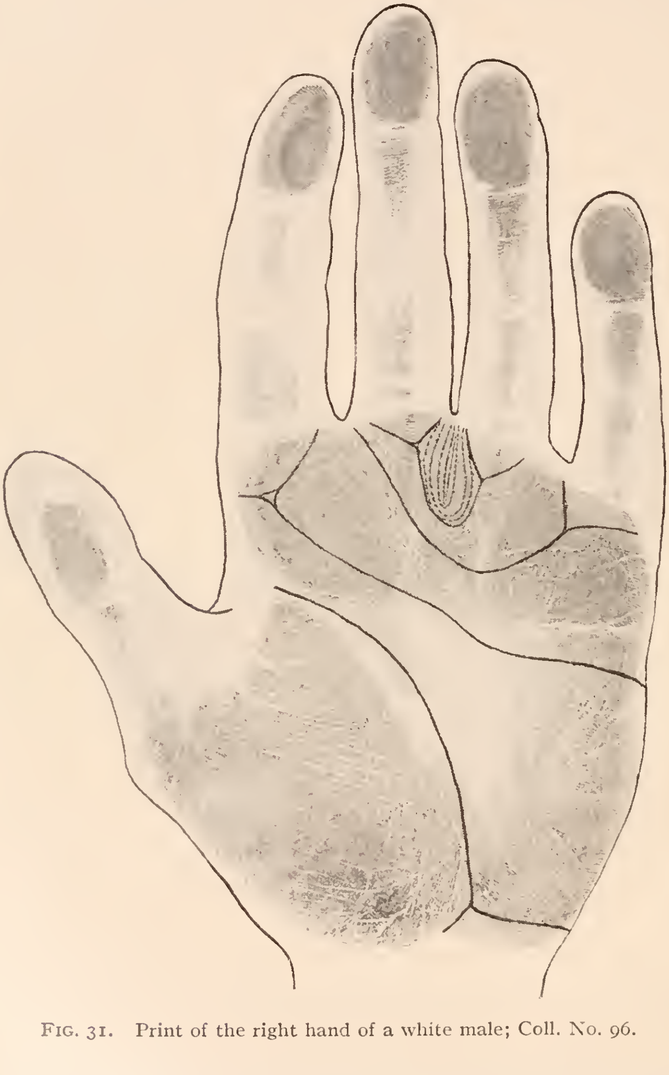


Fig. 31. Print of the right hand of a white male; Coll. No. 96.

VI. The Heritability of Friction-Skin Characters.

A casual inspection of the palm and sole prints of any family where two or more generations are represented, and with several children, indicates that the special configuration of the friction-skin ridges is to some extent a character, or series of characters, transmissible by heredity from parent to offspring. To show this, two pairs of palm prints are here presented, in each case that of a father and son, taken from two unrelated white families.

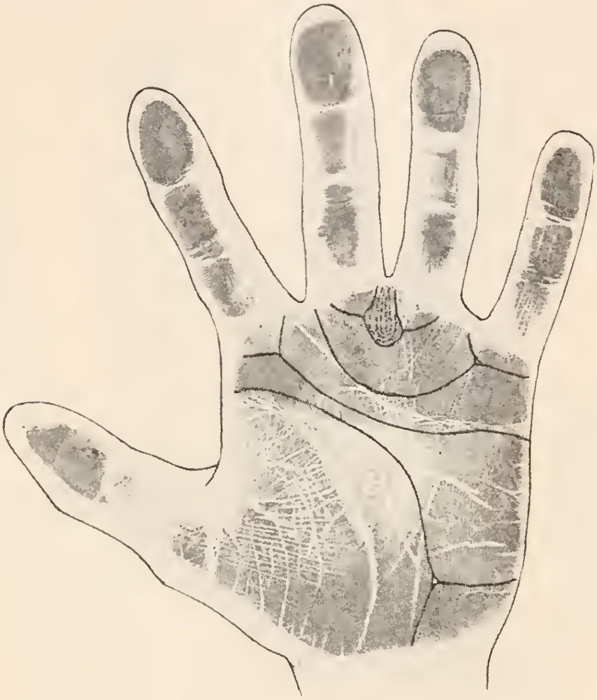


Fig. 32. Print of the right hand of the six-year-old son of the foregoing, showing a strong hereditary influence.

In the first of these (Figs. 31 and 32) the type presented is no unusual one, yet it is interesting to see how the details of the father are faithfully copied by the smaller hand of the son. The same degree of similarity is shown by the hands of the other side, although the two sides are not alike. Otherwise, so far as may be seen in a boy of six, the son does not especially resemble the father. It is also interesting to note that neither the hands of the mother, nor of the mother's sister, are in any way like those of the father and son.

The second pair (Figs. 33 and 34) is of still more value as an illustration of direct inheritance, for here the palm of the father presents two unusual features: (1) the reduction of line *C*, with its triradius, as described above, and (2) the narrowed, and nearly suppressed, hypothenar loop, with two triradii, and with its pattern a loop

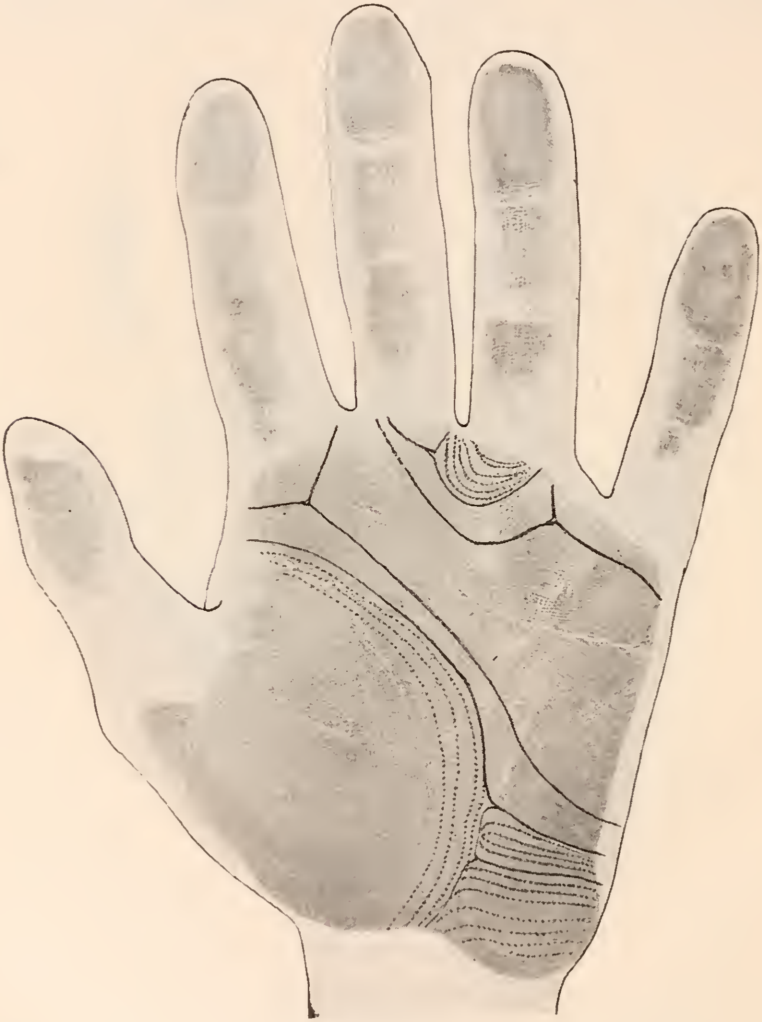


FIG. 33. Print of the right hand of a white male; Coll. No. 99.

opening to the ulnar margin; yet each of these unusual characters is faithfully reproduced in the little hand of his two-year old son. The first of these characters occurs in about 18 per cent. of all cases, although not always to the same degree, as has been stated above, but the type of hypothenar here shown is much rarer, and one might have to look over several hundred sets of palm prints before meeting with a similar one. This type is given by Miss Whipple (1904) as *B*, Fig. 47, p. 349, where its development from a more typical pattern is explained. As a test of its frequency of occurrence the hand prints of 100 individuals from my

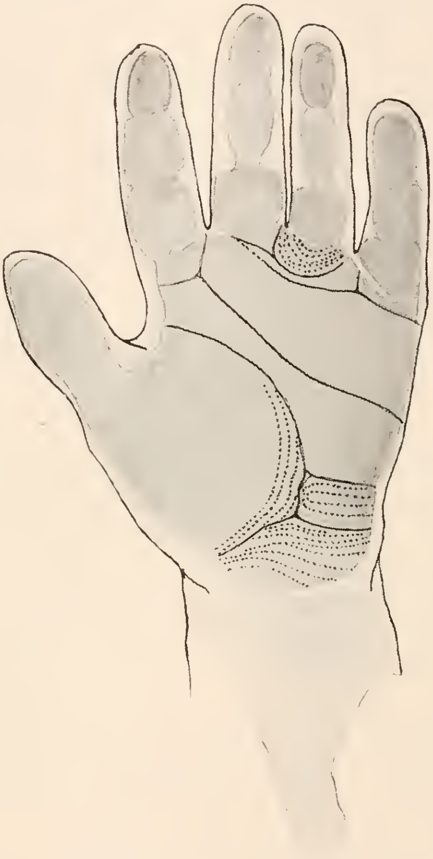


FIG. 34. Print of the two-year-old son of the foregoing, showing a strong hereditary influence, involving in this case two unusual features.

collection were counted, and in eight hands only was the general type of pattern found, that is, eight out of two hundred, or four per cent., and each of these differed more in the arrangement of triradii and other details, than do these of father and son. It would be safe to say that the chance of finding two cases with

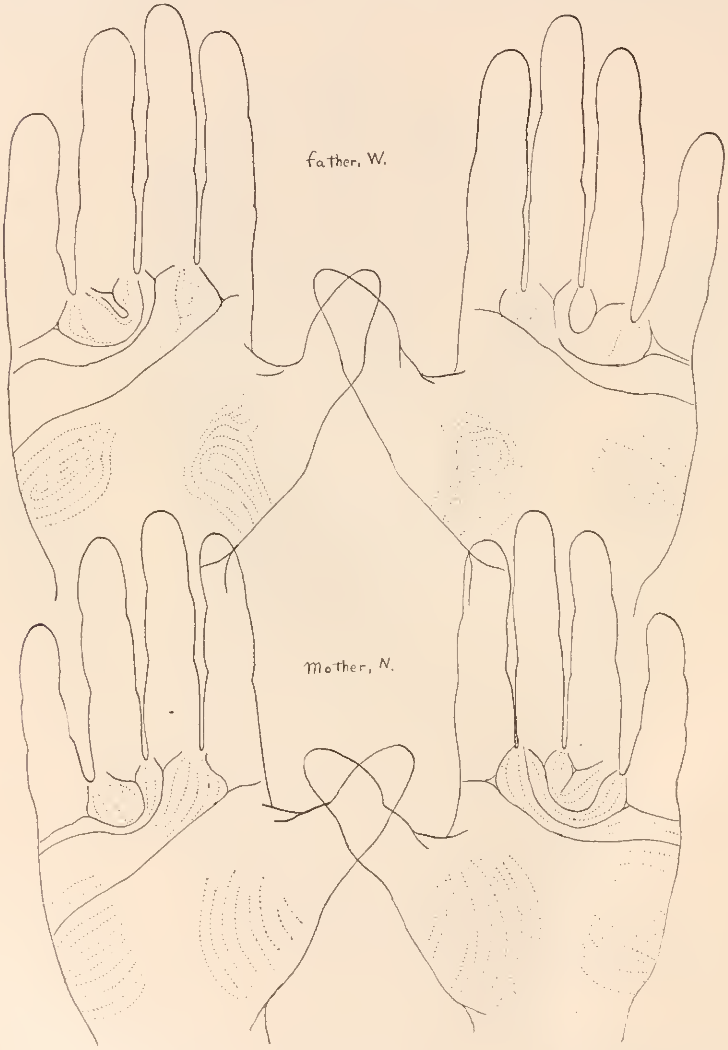


FIG. 35. Tracings of the hand prints of the two parents, *W* and *N*, of the H—— family.

hypothenar patterns of the same type and with the details so similar would be at most one in a hundred, and probably much less. Multiplying this rough estimate (1 in 100) by the chance of 1 in 12, the figure already obtained for the occurrence of a complete suppression of line *C*, and we get 1 in 1,200 for the probable occurrence of these two characters in combination, relying upon chance alone.

The other hands of this same pair, the lefts, correspond almost as closely, with a like suppression of line *C*, and with the hypothenar in each reduced to a single triradius, and no other trace of a pattern. Here the mother's hands also were both of this latter type with respect to the hypothenar, but the main lines are entirely different.

The results from such isolated instances, however, show merely that *in the friction-skin configuration we are dealing with heritable characters*, and in themselves show little more. A more detailed line of inquiry is offered by the study of a large family, where the parents belong to distinctly different types, and where the children are numerous. Such a family is that of H — from my collection, where the material consists of the complete prints (palms and soles) of the father, *W*, the mother, *N*; and six children, *G*, *V*, *M*, *C*, *F*, and *E*, together with the palm prints of the father's three sisters, *Fa*, *Mi*, and *Lo*, which serve as valuable collateral evidence. Of the six children *F* is a boy, the other five are girls.

The hands of the two parents, *W* and *N*, are of distinctly different types, that of *W*, the father, being very complex, with traces at least of all five palmar patterns, while the palms of the mother, *N*, are unusually free from such special features, and show only an included loop between lines *C* and *D* of the left hand, possibly a fourth interdigital, also a loop across the free end of the rudimentary line *C* of the right, the condition designated in a formula by the digit 8.

These two opposite types, mated, have produced six offspring, in which the presence or absence of the five palmar patterns are presented here in tabular form:

The presence of a pattern is represented by a ×, the absence by —, and a rudiment by r. The two designations in each column

Name.	Thenar.	First Id.	Second Id.	Third Id.	Fourth Id.	Hypoth.
G.	× ×	× ×	– r.	× ×	– –	– –
V.	× ×	– –	– –	× ×	– –	– –
M.	× –	– –	– ×	× ×	× ×	– –
C.	× ×	– –	– –	× ×	r. –	– –
F.	× ×	× ×	× ×	× ×	× ×	– –
E.	× –	× –	– –	r. ×	× –	r. –

are for the two hands, the designation at the reader's left being the left.

Aside from the various interdigital patterns, which occur in about three fourths of the cases (36 out of 48), and in one case (third) are practically universal, there will be noticed the remarkable result in the case of the two most prominent patterns, *thenar* and *hypothenar;* the former, a somewhat rare pattern, which occurs in only about 4 per cent. of the white race, is present here in 10 out of the 12 hands (83.3 per cent.), while the much commoner hypothenar, occurring in 20 per cent. of hands of the white race, and found also in both hands of the father, is actually present, aside from a rudiment, but once in the 12 (8.3 per cent.). Now as the father has both thenar and hypothenar patterns upon both hands, and as the mother has neither, it would suggest that possibly the thenar region may be here controlled by the male parent, and the hypothenar by the female.

As the thenar pattern is usually so rare, *its almost universal occurrence in this family is without doubt due to direct inheritance from the father.* For more careful examination the cases are here figured. The usual, or typical, thenar pattern is a double one, like that of the father's left, or *G's* left, with two loops turned away from each other, but with the loops themselves in contact. Morphologically the small, upper loop is in reality the first interdigital, while the lower loop is the thenar proper. This family, however, although the typical form is not wanting, shows a strong tendency to suppress the upper, or first interdigital, loop, and in some cases to modify the lower, the course of degeneration following two lines, as follows:

Series 1.—*W*, left, *F*, right; *V*, left, *C*, right.

Series 2.—*W*, left, *M*, left; *V*, right, *E*, left.

Only upon two palms out of the 12, *M*, right and *E*, right, is a pattern entirely lacking in the thenar region.

It thus seems certain, as already said, that there is here an unmistakable case of the inheritance of a thenar pattern from the male parent, and, except for the failure of this in two of the twelve cases, the character acts like a Mendelian dominant. On this basis the failure in these two cases may be explained by (1) that the father himself is heterozygous, or (2) that the character is not a unit character. As for the first the collateral evidence of the father's three sisters is to the point, for in these *Fa* and *Mi* have good thenar patterns, on both hands. In the third sister, *Lo*, the extreme smoothness of the skin produced an almost illegible print, although upon the left hand a part of a double pattern, the first interdigital loop, is easily made out, rendering the presence of a thenar figure upon this area absolutely

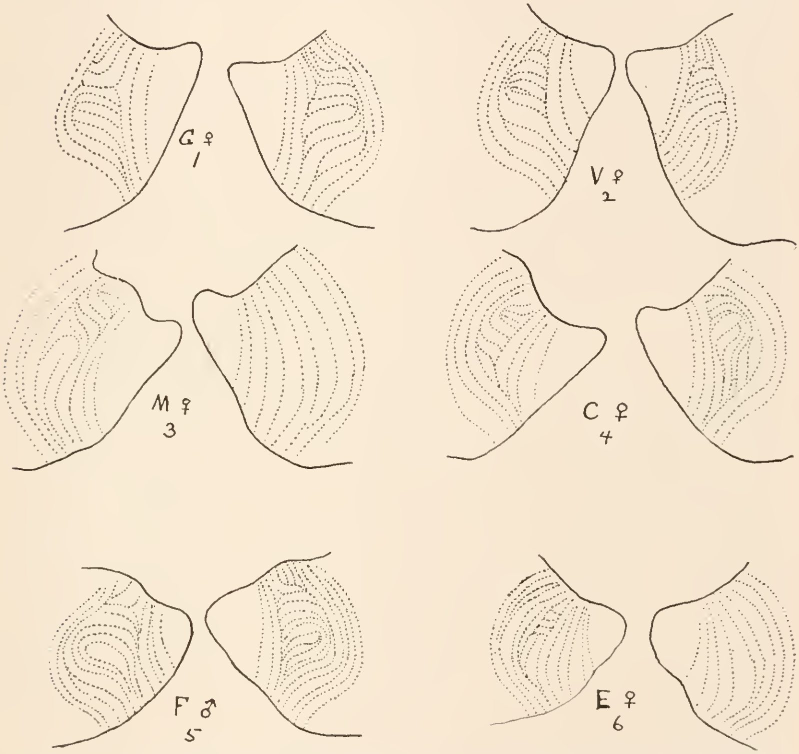


FIG. 36. Tracings of the thenar region in each of the six children of *W* and *N*, the individuals illustrated in the preceding figure. The fifth child, *F*, is a son, the rest daughters.

certain, while it is possible to interpret in a similar way the faint indication of certain lines on the thenar area of the right. It is also noteworthy that the type of pattern upon the right hand of *Fa*, a quite unusual type, is practically identical with that which her niece *C* has upon the same hand. This universality of the thenar pattern in four individuals of the father's generation (8 hands) would suggest dominance.

Concerning the second possibility, that the character here examined, the thenar pattern, is not in itself a Mendelian unit, but is conditioned by two or more factors, this is rendered probable by the many stages of modification, or degeneracy, represented by the hands here represented, eight in the older generation, and ten in the younger. It suggests the presence of a factor tending to reduce the pattern to a series of parallel lines that follow the strong line of flexion bounding the thenar region, the "Line of Life" of the palmist; and the presence of the ancestral thenar pattern, or some stage in its reduction, in so many hands of the H— family, may be due to the absence of this latter factor fully as much as to the presence of a positive thenar pattern unit.

Quite aside from all this, the fact that such grades in the reduction of a pattern may exist, and that for a given pattern there may be found several series of them[1] is in itself a phenomenon of heredity at present inexplicable, for, *while in such a series we may find every step in a definite and continuous process, the steps themselves do not occur in ontogenetic development, but each step arises in an individual embryo precisely in the form which that individual shall retain throughout life.* Since, now, this process of the degeneration of the ridge formation is from a complex pattern to a series of parallel lines; and since the goal sought in this process is in countless details, precisely what would result from the action of external forces upon yielding ridges, as such authors as Miss Whipple and W. Kidd have abundantly shown, we have the startling phenomen of *a Lamarckian environmental influence of a mechanical nature, which, while having no visible effect upon the soma, so modifies the germ-cells that the effect becomes transmitted to the descendants, and that probably by Mendelian methods!* There has thus developed a long series

[1] Cf. Miss Whipple, 1904, pp. 339–352, esp. Fig. 48, p. 350.

of generations, with its beginnings somewhere in the Simian line, the individuals of which, though with many a retrogression, have progressed steadily from a series of complicated patterns, developed in association with raised pad organs, to a simple condition of parallel ridges, more or less conformable to the principal lines of flexure.

Expressed in another way, the results of the study of friction-ridges, especially in connection with their possible heritability, may be collected into a series of definite statements, as follows:

I. Each friction-ridge pattern presents, by taking a large number of human individuals, every stage in the process of development, from a whorl of concentric lines with a definite number of embracing triradii, to a condition in which core and triradii are completely lost and the surface is covered by simple parallel ridges, straight or slightly curved.[1]

II. From comparative study of other Primates it is plain that the course of evolution has been from the involved condition, the whorl, which is distinctly Simian, through every stage in the reduction of the pattern, to its final complete effacement.[2]

III. A given pattern shows as a rule more than one way in which it may degenerate, often two or three, and each way may be shown by complete series of instances taken from individual cases, and leading to the same final result.[3]

IV. The ridges forming a given pattern are first seen in an embryo of approximately four months, since which age there is no indication of change throughout life. This has been absolutely proven in the case of individuals after birth by separate observations taken upon the same person, and in a number of instances these observations have been separated by a period of fifty or more years; in the embryo the ridges appear in a definite form, and there is no indication of later modification or of provision for it.[4]

---

[1] Miss Whipple, 1904.

[2] Miss Whipple, 1904.

[3] Miss Whipple, 1904, pp. 343-354.

[4] The occurrence of ridge rudiments, occasionally found lying between normal ridges, and reduced often to broken lines without sweat-pores, suggests the possibility that in an early embryonic stage these may have been as large as the others, and that they may have become pushed out of existence, or *suppressed*, owing to some mechanical pressure during ontogeny. Of this we have no proof,

V. It follows, therefore, *that the modifications of the original typical patterns*, as given in II. and III. above, *must be affected through modification of the germ-cells, which determine at each fertilization the form of pattern for the new individual.* These patterns are often atavistic, that is, a primitive pattern may occasionally appear, but in the main the progress has been a decided one, so that the majority of men show a nearly complete effacement of the original complicated condition.

VI. In each given area *the characteristic ridge direction* of a typical human hand *is precisely that to which the ridges would be pushed or pressed if considered plastic and capable of being acted on directly by the objects which naturally come into contact with them in the ordinary uses of the hands and feet.* That is, the ridges are everywhere arranged to run at right angles to the prevailing direction along which contact objects would push or slip. In the hand, which is constantly grasping cylindrical objects, such as boughs, handles of instruments, ropes, and numberless similar things, the ridges of the distal half of the palm tend to lie straight across it, a position which, in the highest degree possible for man, is expressed by the formula 11·9·7·5. It is thus significant that *in the right hands of all human races this formula is found in 22–25 per cent. of the cases*, while in left hands this formula forms only 4 per cent. of the cases.[1] That the same percentages hold among such diverse human races as Whites, Negroes, and Maya Indians marks this as *a natural human tendency, and suggests that this result has been gained since the adoption of right-handedness.*

VII. Finally, (1) since the configuration of the friction-ridges is directly heritable, probably in conformity to the laws of Mendel; (2) since the countless elements involved in the ridge arrangement are precisely similar to what would be produced in

---

and it is not very likely, yet, until otherwise explained, their occurence prevents the dogmatic assertion that no change can absolutely take place after the ridges are first laid down. Concerning a modification of this sort after birth, this is still less likely, and may be practically denied. The author is in possession of a series of prints of the right forefinger of a child, beginning with the second year, continued at approximately two-year intervals until the age of twelve, and still being collected. In this there is an unusual number of these "suppressed" ridges, yet in the interval covered by the series there has not been the slightest change in any of them.

[1] Wilder, 1904, *Amer. Anthrop.*, Vol. 6, p. 279.

a plastic material by the direct application of the most usual objects, acting in the most usual way, and (3) since no modification of an individual plan of configuration seems possible during ontogeny, or as a result of continued action upon an individual, *we find ourselves confronted by the phenomenon of a complex series of arrangements, each corresponding exactly at all points with the direct result of the action of constantly applied external forces upon plastic structures; and yet, although these external forces are constantly applied, and are without the slightest visible result upon the individual, positive results, cumulative in their effects, are actually transmitted from parent to child. Furthermore, these results must be transmitted by the germ-cells, and are probably in accordance with the Mendelian laws.*

One could hardly find a more startling combination of principles than the above, supporting at the same time the ideas of Lamarck, Mendel, and Weismann, with Darwin left entirely out, since with the reduction in the size of the friction-ridges and the increase in the size of the body, they can be of no real selective value above the Cercopithecidæ; yet from a long and careful study of a large amount of data (perhaps a thousand hand prints, including the most varied human races, studied during fifteen years) every one of the above conclusions seems amply substantiated. The facts stand written upon the palmar and plantar surfaces of mankind; the detailed record of their physical exertions and that of a long line of ancestors; these records are inherited with numerous individual modifications, so that, except in the case of duplicate twins, the offspring of the same two parents are never quite alike; it is easily possible to arrange these individual modifications in developmental series, presumably corresponding to their evolution, and, when so arranged, they follow closely the theoretical series of modifications which would be induced by direct influence from the usual external mechanical forces, in case these structures were sufficiently yielding to allow it.

Such are the conclusions which, from my study of friction-skin configuration, I have reached at present. They seem inevitable, and if so, are exceedingly important; and it is because they seem so that I wish to make an appeal for others to undertake to investigate the same subject. In the study of friction-skin may be found taxonomy, morphology, ethnology, genetics, as well as the practical application to problems of individual identification. It must be confessed that there is at first a large amount of detail which must be acquired, as well as a certain amount of nomenclature. The bibliography, however, if that which deals with the special work on apical patterns ("finger-prints") be disregarded, is not large, and as it is for the most part recent it can be readily obtained. Unlike most biological material, that concerned here is readily obtained, and easily kept, a series of prints being for most purposes more convenient for study than the actual objects; the prints, too, are more accurate records of the facts than are any drawings or even photographs.

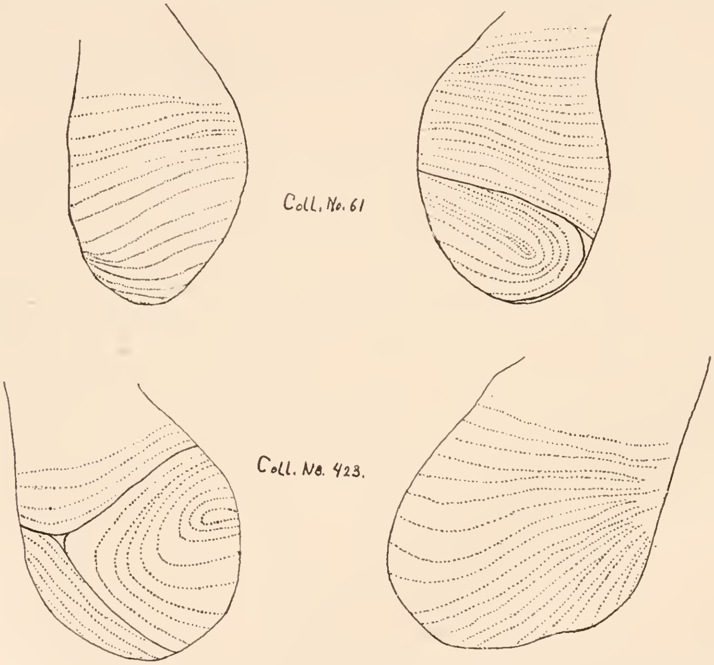


FIG. 37. Tracings of the only calcar patterns in my entire collection, outside of the two families considered below.

We who have been at work along this line are in great need of collaborators. We are firmly impressed with the belief of the biological importance of the field, and feel that many weighty conclusions are to be drawn from it. It is with the idea of facilitating study that I give here, as the final paper in this series, a bibliography of the subject, which should make the study at once available to those who care to interest themselves in it.

Still more convincing than the inheritance of the thenar pattern in the hands of the H— family is that of the calcar pattern in a family which I may designate as Wh—. This pattern is the rarest of all friction-skin patterns known in man, and has been found once by Schlaginhaufen,[1] once by Féré,[2] and, outside of this family, twice by myself (Fig. 37). In the sole prints of 100 Liberian soldiers, collected by Starr, there is not an instance of it, and Schlaginhaufen states in 1905, including all the observations to date, that it has never been reported in any non-European race.

As it appears upon the tread area this pattern is in the form of a simple loop, with its apex fibulo-proximal and with its opening directed obliquely towards the tibial and distal directions (Fig. 23). This loop is usually accompanied by a triradius placed distally to it and on the fibular side, but this does not always appear upon the tread area, and in general the portion thus included gives the impression of being a part of a more complete figure, the remainder of which should lie farther up on the side of the foot. In fact Schlaginhaufen, who seems to be the only one who has followed this pattern beyond the tread area, has traced the loop in his case over the hollow on the tibial side of the foot and reports that there the lines of the loop converge, turn about, and form a second loop facing the other way and accompanied by a second triradius, so that the entire figure, thus completed, becomes a long drawn out spiral with two loops, one of which lies within the tread area.[3]

Occasionally, though not often, prints are found in which the lines which run across the heel region of the tread area diverge from one another at a point near the tibial margin of the print, or perhaps spread out along this border from a shorter portion of the opposite side, and as this appearance often occurs where there is some connection with a typical calcar pattern (either on the other foot of a subject possessing this pattern or upon those related to such a subject) it gives probability to the surmise that we have here the vestige of a calcar pattern (Fig. 42). If this be so it suggests that the tendency to form a calcar loop is much commoner than the realization, and that a certain configuration, although reduced by some unfavorable influence, is very hard to entirely eradicate.

---

[1] 1905; Fig. 178, p. 100.

[2] 1900, Les lignes papillaries de la plante du pied, 1900; Fig. 18, p. 615.

[3] "Ich verfolgte jedoch die Crura des Sinus in tibiodistaler Richtung und gelangte zu folgenden Leistenbild. Gegen die Cavität des Fusses convergieren die Schenkel, um einer über den anderen hinweglaufend umzubiegen; d.h., wir haben eine langgestreckte, linksgewundene Spirale. Ihrem tibiodistalen Ende liegt ein zweiter Triradius an, dessen Lage in die Fusshöhlung fällt." Schlaginhaufen 1905, pp. 100–101.

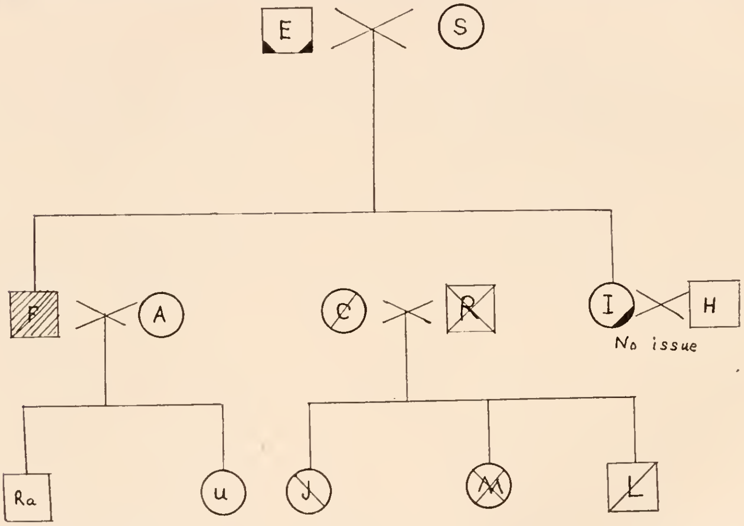


FIG. 38. Diagram of the relationships of family Wh———. The squares represent males, the circles females. A calcar pattern on the right heel is indicated by a diagonal line drawn through the square or circle, from the right above to the left below; one on the left heel by the opposite diagonal. Where both diagonals are used there are two calcar patterns. A divergence, indicating a rudiment of the pattern, is indicated by a black lower corner on the side of the divergence. A shaded square or circle indicates that, through decease or otherwise, prints are not available.

To proceed now to the conditions obtaining in this family (Wh—). The maternal grandfather *E* and the grandmother *S* show the usual type of heel, save that in the former there is in each foot a noticeable divergence of the lines towards the inner margin, which although of no especial importance in itself, may in connection with the descendants have some significance. (Fig. 42.)

This pair had one son and two daughters, one of which, the daughter *C*, possesses a calcar loop upon the right heel, and a divergence upon the left. The other daughter, *I*, shows on the right heel a noticeable divergence, but no loop, while the left heel is normal. The son *F* is deceased, but his wife is normal,

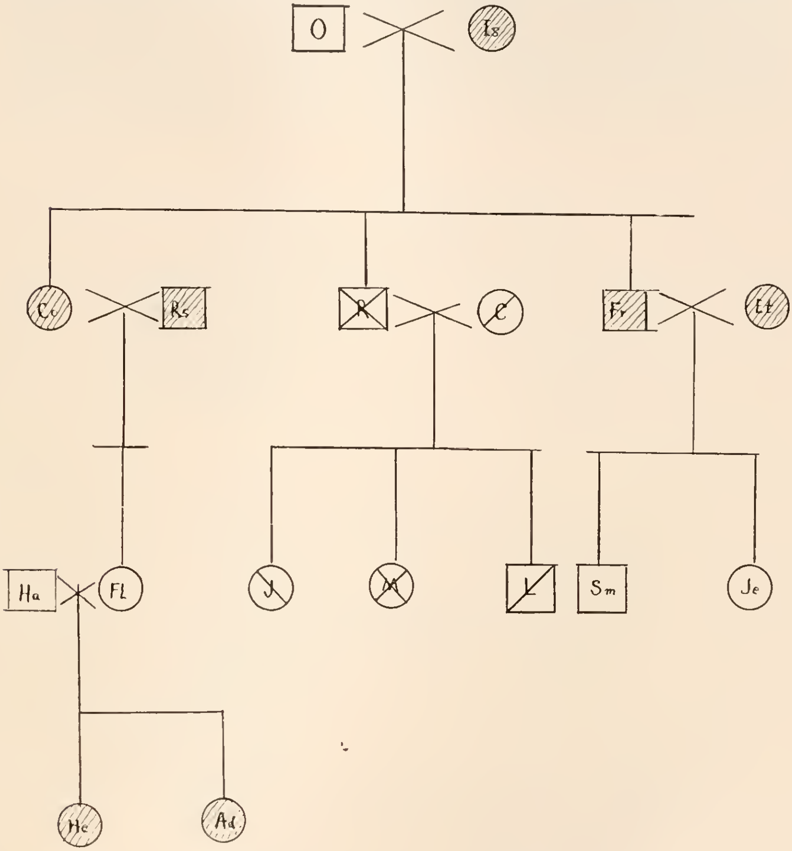


FIG. 39. Diagram of the relationships of the family Wo———, that of the husband, *R*, of the double mating. Symbols as in the previous figure. The sister, *Co*, is deceased, and her husband, *Rs*, is not now available, but their daughter, *Fl*, is normal. Her two children have not yet been investigated. I have not the prints of the brother *Fr*, or of his wife, *Et*, but again the heels of the two children, *Sm* and *Je* are normal. The source of the double calcar pattern of *R* remains thus far unknown.

and neither of his two children, *Ra* and *U*, show anything unusual in the heel markings.

The daughter C, who possesses one calcar loop, by a most extraordinary coincidence, married an unrelated man, R, who possesses a typical calcar loop on both feet, a case unheard of except in this family, and their three childrem J, M, and L, all possess calcar loops, J (female) upon the left, M (female) upon both, like the father, and L (male) upon the right, with a marked divergence on the left. In the right foot of J there is also some divergence. These results may be tabulated as follows, using an *X* for a calcar loop, and an *r* for a divergence. The two symbols used for each individual signify the two feet, in their natural position. The enclosed symbols represent males.

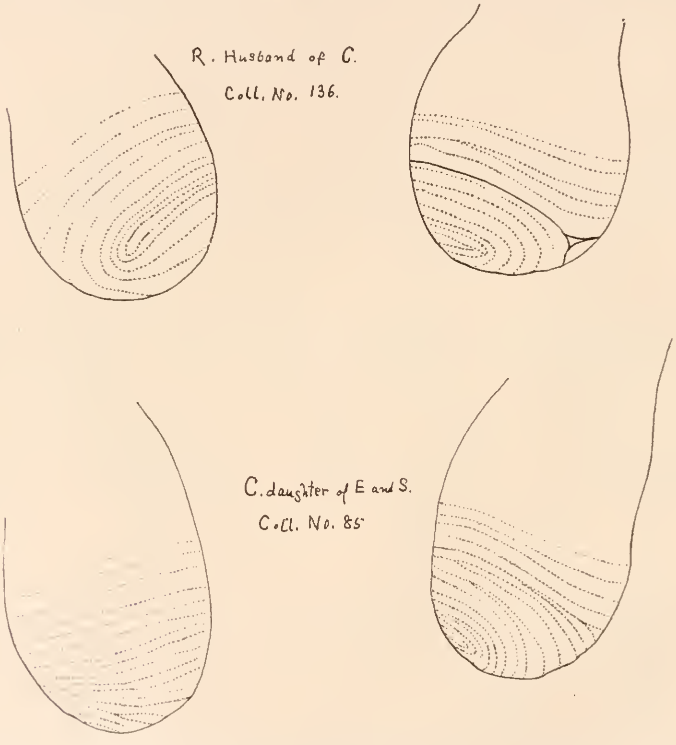
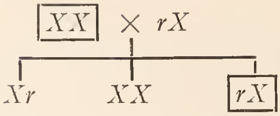


Fig. 40. Tracings of the heel patterns of the mating *R* × *C*, the first with double calcar patterns, the second with a single one, the right.


XX
× rX
Xr
XX
rX

Concerning the source of the calcar loops in the parents, the father of *R* was investigated and found to have quite normal heels; the mother of *C* is also normal, but the father, *E*, shows a good divergence upon both heels. Probably, then, *C* obtained her calcar loop from her father rather than from her mother, and in the same way it may be surmised that the husband *R* got his from the side of his deceased mother *Is*, but for lack of further

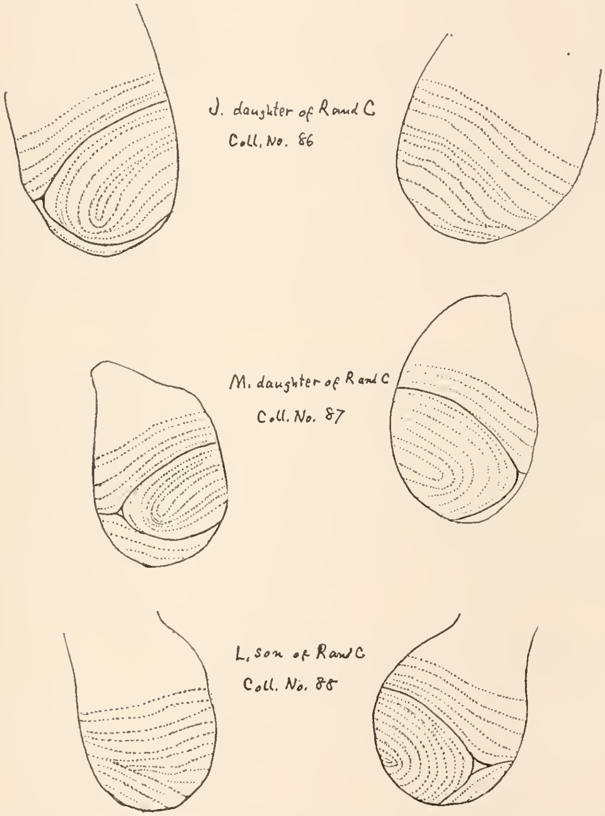

J. daughter of R and C
Coll. No. 86
M. daughter of R and C
Coll. No. 87
L, son of R and C
Coll. No. 88

FIG. 41. Heel patterns of the three children of *R* and *C*, shown in the previous figure. The oldest, *J*, has a calcar pattern on the left foot; the second, *M*, on both, and the third, *L*, on the right.

data this question cannot be definitely decided. Certain brothers and sisters of most of the four grandparents, *E*, *S*, *O*, and *Is*, are still living, but to obtain the necessary data from them would be difficult; there is more to expect from the coming generation, the offspring of *J*, *M*, and *L*, and of their cousins, *Sm* and *Je*, *Ra*, and *U*, but for that several years must elapse.

Leaving the past and the future as open questions, we return to the facts here presented, and these are, *that a man with a calcar loop on both feet married a woman with a loop and a divergence, and that, of their three children, one has a loop on both feet, and two have a loop on one foot and a divergence on the other, the loop being on the right foot in one case and on the left in the other.* When this record is considered in connection with the fact that, aside from this family, but four loops are known to science (that is, in perhaps 1,000–1,500 individuals of all races) *the direct inheritance of this condition by the mating of* $XX \times rX$ *is beyond question.*

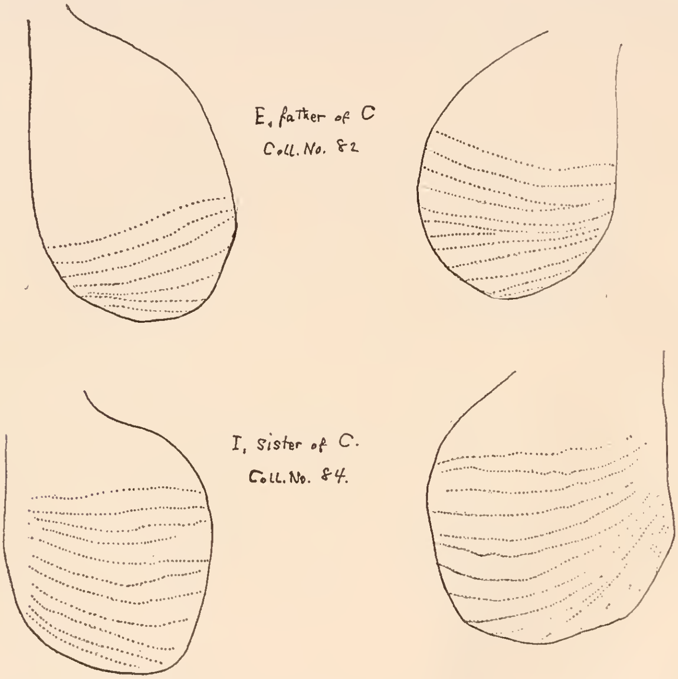


FIG. 42. Heel patterns of the only relatives of *C* which show even a rudiment of a calcar pattern; the father, *E*, and the sister, *I*.

Here there is brought forcibly before the reader the question of *unilateral inheritance, i. e.*, whether a character possessed upon the right side only in the parent may cross over in the offspring, and appear on the left side, or whether each side inherits independently. The mother *C* has a loop upon the right foot, and a divergence only upon the left, and the same condition obtains in her son *L*. In the daughter *J*, however, these conditions are reversed, and the right loop in both father and mother fail to govern the right heel of *J*. The loop which *J* possesses upon her left foot may have been inherited from the father, as he has a loop on each foot, but does the loop upon her mother's right foot have any influence? Facts thus far, in all the families studied, tend to show that inheritance does not cross from one side to the other, but we are yet a long way from stating this definitely, or even as a plausible hypothesis. The two definite points that appear as the result of this investigation are (1) *that the calcar loop is heritable, and* (2) *that its presence upon the right foot of both parents does not compel its appearance upon the same foot of the offspring.*

VII. Bibliography of Friction-Skin Configuration.

This list is intended to be complete on the subject of the friction-skin configuration of the palms and soles. On the apical patterns it makes no attempt to be exhaustive, as the subject is now in the hands of the police department of all civilized countries, and has been largely exploited for practical purposes of personal identification, developing a large mass of literature hardly morphological in the technical sense.

There is also little or nothing upon the structure of the skin as such, on its development or histology, or on the innervation, which has been especially a subject of investigation by psychologists.

At the end there is appended a list of the published investigations of Newman and Patterson on the subject of polyembryony in the armadillo, extensively referred to in Section IV. of the present paper. This subject, as it relates to scute and band anomalies of the carapace, and their inheritance by twins and other duplicate individuals, is of special interest here.

D'Abundo.

'91 Contributo allo studio delle impronte digitali. Archivio di Psichiatria. Pisa.

'94 Le impronte digitali in 140 criminali. Riforma medica.

Alix, M.

'67 Recherches sur la disposition des lignes papillaires de la main et du pied. Ann. des Sci. Nat., T. VIII., pp. 295–362.

'69 (Same title.) Ann. des Sci. Nat., T. IX., pp. 5–42.

Bruhns, Fanny.

'10 Der Nagel der Halbaffen und Affen. Morph. Jahrb., Bd. XL., pp. 501–609. (Ridges of finger-tips incidentally mentioned.)

Daae, A.

'94 Ueber Fingerabdrücke und deren Verwendung zur Identitätsfeststellung, verglichen mit Bertillon's Anthropometrischen System (Uebersetzt von Teichmann). Zeitschr. für schweizerisches Strafrecht. Jahrg. VII., p. 317+.

Dixey, F. A. On the Epidermis of the Plantar Surface and the Question of Use-inheritance. Rep. 64th Meeting British Assn. Adv. Sci. (This treats of the thickness of the epidermis on the fetal sole.)

Evatt, E. J.

'06 The Development and Evolution of the "Papillary" Ridges and Patterns on the Volar Surface of the Hand. Journ. Anat. and Physiol., Vol. XLI., pp. 66–71.

Faulds, H.

'80 On the Skin Furrows of the Hand. Letter in Nature, Vol. XXII., p. 605. (This remarkable letter, written from the Tsukiji Hospital, Tokio, anticipates every direction of the later investigation; comp. anat; ethnol; indiv. variations; heredity, etc.)

Faulds, H.

'94 On the Identification of Habitual Criminals by Finger-prints. Nature, Vol. L. (F. claims priority to Sir Wm. Herschel, because of his letter of October 28, 1880, the previous title. Herschel's letter to Nature is dated November 25, 1880.)

Féré, Ch.

(This author has written numerous short papers on the apical patterns of both fingers and toes. The most important citations are the following:)

'91 (Finger and toe patterns.) C. R. soc. biol., Tome 43, pp. 497–506.

'92 (With Batigny, on the same subject.) C. R. soc. biol., Tome 44, pp. 802–806.

'93 (Finger and toe patterns.) Journ. de l'Anat. et de la Physiol. Année, 29, pp. 223–237.

'95 (Sensibility of the balls of the fingers.) C. R. soc. biol., Tome 47, pp. 657–660.

'96 (Finger patterns in relation to function.) C. R. soc. biol., Tome 48, pp. 1114–1116.

'98 (Finger patterns in relation to functional aptitude of the hand.) C. R. soc. biol., Tome 50, p. 837+.

'00 (Hands and finger patterns of certain monkeys.) Journ. de l'Anat. et de la la Physiol. normales et pathol. de l'homme et des animaux. Ann. XXXVI., pp. 255–267.

'00 (Papillary lines of the palm of the hand.) Journ. de l'anat. et de la physiol., Ann. XXXVI., pp. 376–392.

'00 (Papillary lines of the sole of the foot.) Journ. de l'anat. et de la physiol., Ann. XXXVI., pp. 602–618.

'00 (Imprints of palms and soles.) C. R. soc. biol., Tome 52, pp. 641–643.

'05 (Finger prints of psychopathic subjects.) Journ. de l'anat. et de la physiol., Ann. XLI., pp. 394–410.

'06 (Papillary lines of the heel.) C. R. soc. biol., Tome 61, p. 44.

Forgeot, Réné.

'91 Les empreints latentes. Thése; Lyons. (Also in same year, in Bull de la soc. d'anthropol. de Lyon, Tome X., p. 189.)

Frécon, André.

'89 Les empreintes en general. Thése, Lyons.

Fülleborn, Fr.

'02 Beiträge zur physischen Anthropol. der Nord-Nyassaländer. Berlin.

Galton, Sir Francis.

'86 (In Science, Vol. VIII., p. 166 and p. 212.)

'88 Personal Identification. Journ. Roy. Inst.

'88 Personal Identification and Description. Nature, Vol. 38, p. 201.

'90 Patterns in Thumb and Finger Marks. Proc. Roy. Soc., Vol. 48, p. 455.

'91 Patterns in Thumb and Finger Marks. Phil. Trans. Roy. Soc., Vol. 182, pp. 1–23.

'91 Method of Indexing Finger Marks. Proc. Roy. Soc., Vol. 49, p. 540. (Also in Nature, Vol. 44; p. 141.)

'91 Identification of Finger Tips. Nineteenth Cent., Vol. 30, pp. 303–311.

'92 Finger Prints. MacMillan, London.

'92 Imprints of the Hand, by Dr. Forgeot of the laboratoire d'anthropologie criminelle, Lyon. Journ. Anthropol. Inst., Vol. XXI., pp. 282–283.

'93 Identification. Nature, Vol. 48; p. 223.

'93 Finger Prints in the Indian Army. Nature, Vol. 48, p. 595.

'96 Prints of Scars, Nature, Vol. 53, p. 295.

'96 The Bertillon System of Identification. Nature, Vol. 54, p. 569.

'99 Finger Prints of Young Children, Meeting of the British Assn., Dover, pp. pp. 868–869.

'99 Finger Prints. Journ. Anthropol. Inst., Vol. 29, p. 199.

'93 The Decipherment of Blurred and Indistinct Finger Prints. MacMillan, London.

'95 Finger Print Directories. MacMillan, London.

Garson, J. G.

'00 A System of Classification of Finger Prints. Meeting of the British Assn. Bradford, pp. 910–912.

'00 Finger Print Classification. Journ. Anthropol. Inst., Vol. XXX., p. 101.,

Henry, Sir Edward Richard.

'99 Finger Prints as a Method for the Identification of Criminals. Nature, Vol. 61, p. 42.

'99 Finger Prints and the Detection of Crime in India. Meeting of the British Assn., Dover, p. 869.

'00 Classification and Uses of Finger Prints, London. (This is the standard book used in police identification bureaus the world over. There are numerous later editions both in England and in the United States.)

Hepburn, David.

'93 The Integumentary Grooves on the Palm of the Hand and Sole of the Foot of Man and the Anthropoid Apes. Journ. Anat. and Physiol., 1893, Vol. XXVII., pp. 112–130.

'95 The Papillary Grooves on the Hands and Feet of Monkeys and Men. Sci. Trans. of the Royal Dublin Soc., Vol. V., Series II., pp. 525–537. (Reviewed in Nature, Nov. 14, 1895, and in Journ. Anat. and Physiol., Jan., 1896.)

'97 Note on Dr. Harris H. Wilder's Paper, " On the Disposition of the Epidermic Folds upon the Palms and Soles of Primates." Anat. Anzeiger, Bd. XIII., pp. 435–437.

Herschel, Sir Wm.

'80 Skin Furrows of the Hand, Nature, Vol. XXIII.; p. 76. (This note, often taken as the first mention of the subject in recent years, and perhaps as the very first suggesting the use of the ridges in identification, was antedated by a month by the letter of Dr. Faulds to the same periodical. Faulds's letter was published in the issue of Oct. 28, 1880 (Vol. XXII, p. 605), and Herschel's paper appeared in the issue of Nov. 25 of the same year.)

'94 Finger Prints. Nature, Vol. LI., p. 77.

Johnson, R. H.

'99 Pads on the Palm and Sole of the Human Fetus. Amer. Nat., Vol. 33, pp. 729–734.

Kidd, W.

'03 On Imbrication of the Papillary Ridges in Man. Journ. Anat and Physiol., Vol. XXXIX., pp. 413–416.

'05 The Papillary Ridges and the Papillary Layer of the Corium in the Mammalian Hand and Foot. Journ. Anat. and Physiol., Vol. XLI., pp. 35–44.

'07 The Sense of Touch in Mammals and Birds, with Special Reference to the Papillary Ridges. London, A. and C. Black, 176 pp. (The title of this book is misleading; in reality it treats mainly of the friction ridge configuration in the Primates, and gives good illustrations and descriptions of individual Primate palms and soles. Review by Inez Whipple Wilder in Science, Vol. XXVII., 1908, pp. 582–585.)

Klaatsch, H.

'87 Ueber die Morphologie der Tastballen. Anat. Anz., Bd. II., pp. 400–401. (Prelimin. Commun.)

'88 Zur Morphologie der Tastballen der Säugertiere. Morph. Jahrb., Bd. XIV., pp. 407–435. (The first morphological consideration of mammalian pads.)

Kollmann, A.

'83 Der Tastapparat der Hand der menschlichen Rassen und der Affen in seiner Entwickelung und Gliederung. Leipzig, pp. 1–75.

'85 Der Tastapparat des Fusses von Affe und Mensch. Archiv für Anat. u. Physiol., Anat. Abteil., pp. 56–101.

Kolossoff, G. and Paukel, E.

'06 Versuch einer mathematischen Theorie der Hautleistenfiguren der Primaten-Palma und Planta. Morph. Jahrb., Bd. XXXV., pp. 697–708.

Kollman,.

'00 (Discussion concerning impressions of finger patterns on the pottery of the

Lake-dwellers, in Correspondenzblatt d. deutsch. anthropol. Gesell., No. 10, Berichte der XXXI. Versamml. in Halle.)

Loth, E.

'09 Dzisiejszy stan wiedzy o filogenii stopy ludzkiej. [The present condition of our knowledge of the phylogeny of the human foot.] Proc. Soc. Sci., Warsaw, II., 8, 1909.

'10 Przyczynek do poznania przebiegu ukladów listewek skórnych na stopie dloni polaków. [Contribution to the knowledge of the arrangement of the integumental ridges on the palms and soles of the Poles.] Proc. Soc. Sciences, Warsaw, III., 4, 1910.

'10 Anthropologische Untersuchungen über das Hautleisten-System der Polen. Zeitschr. für Morphol. u. Anthrop, Bd. XIII., pp. 77–96. (An abridgment of the above, in the German language.)

'13 Zum Artikel des Herrn Prof. Schlaginhaufen; Beobachtungsblatt und Anleitung zur Aufnahme von Hand- und Fuss-abdrücken. Correspondenzbl. d. deutschen Gesell. für Anthrop., Ethnol., u. Urgeschichte. Jahrg. 44, No. 1, Jan., 1913.

Maack, F.

'01 Das Hautleistensystem an den Fingerspitzen. Wissensch. Zeitschr. für Xenologie, No. 7, pp. 6–15.

Malpighi, M.

1686 De externo tactus organo exercitatio epistolica ad Jacobum Ruffium. Londini. (The first known reference to friction-ridge patterns; quoted by Alix, 1867, and by Schlaginhaufen, 1905.)

Martin, R.

'14 Lehrbuch der Anthropologie. Jena, 1914. Cf. pp. 360–367 for methods of formulating palms and soles.

Meisner,

'00 Scherben mit Fingereindrücken. Correspondenzbl. d. deutsch. anthrop. Gesellschaft. XXXI Versamml. in Halle. (Short notice of pits made in pottery of the Lake-dwellers by the finger tips of the potter; farther discussion by Kollmann. Although in this there is no mention of the friction-ridges, the paper suggests possible material for investigation. There is frequent mention in literature of similar impressions.)

Minakata, K.

'94, '95 (Two short letters in Nature, Vol. LI., pp. 199–200, and 274. These present claims of the use of the "Thumb-stamp" by the Japanese prior to Sir Wm. Herschel. Cf. in this connection the references to the latter and to Dr. Faulds, above.)

Morselli, E.

'74 Sulla dispozisione delle linee papillari nella mano e nel piede del Cercopithecus mona. Ann. di soc. di nat. di Modena, Anno VIII.

Niemirycz-Lothowa, Jadwige (Mme. Loth).

'12 O rzadkim przypadku przebiegu listewek skórnych na stopie murzyna. [A case showing a very rare disposition of the integumental ridges on the foot of a negro.] Proc. soc. sci., Warsaw, V., 9, 1912.

Paul, Fr.

'03 Sichtbarmachen latenter Finger- und Fuss-abdrücke. Archiv für Kriminalanthropologie, Bd. XII., pp. 124–129.

'03 Die Kollectivausstellung der Polizeibehörden auf der Städteausstellung in Dresden. Arch. für Kriminalanthropologie, Bd. XIII., pp. 321–323.

Prant, A.

'00 Ueber das Aufsuchen von Fussspuren und Händeabdrücken und ihre Identifizierung. Arch. für Kriminalanthrop., Bd. III.

Purkinje, J. E.

'23 Commentatio de examine physiologico organi visus et systematis cutanei, etc. Vratislav (Breslau). A thesis delivered at Breslau in 1823. This, except for the brief mention by Malpighi in 1686, is the first paper published in Europe, at least, treating of the subject of finger-prints. The original is very rare (a copy in the Surgeon-general's Library in Washington), but the essential parts of it are given in Engl. transl. in Galton's Finger Prints, 1892, pp. 84–88. Cf. also Roscher, 1906, below.

Radl, H.

'95 Fingerabdrücke in Bosnien. Globus, Bd. LXVII., p. 388.

Retzius, G.

'04 Zur Kenntnis der Entwickelung der Körperformen des Menschen während der fötalen Lebensstufen; in Biol. Untersuchungen, XI., pp. 33–76. (Mainly the pads.) Preliminary commun. of this in Verh. d. anat. Gesell., 18th Versamml. Jena, pp. 41–43.

Roscher, G.

'05 Handbuch der Dactyloskopie. Leipzig.

'06 Der Altmeister der Daktyloskopie. Ein Gedenkblatt für J. E. Purkinje. Arch. für Kriminalanthropol., Bd. XXII., pp. 326–335.

Sanctis, S. de, e Toscano, P.

'02 Le impronte digitali dei fanciulli normali, frenastenici, e sordo muti. Atti d soc. Romana di Antropol., Vol. VIII., pp. 62–79.

Schlaginhaufen, O.

'05 Das Hautleistensystem der Primatenplanta mit Berücksichtigung der Palma. I. Morph. Jahrb., Bd. XXXIII., pp. 577–671. II. Morph. Jahrb., Bd. XXXIV., pp. 1–125. This paper, with that of Miss Whipple (1904), appearing almost at the same time, established the principles of comparative morphology involved in the friction-skin configuration, and are hence invaluable to one wishing to go into the subject fundamentally.

'05 Beiträge zur Kenntniss des Reliefs der Planta der Primaten und der Menschenrassen. Korrespondenzbl. d. deutschen Anthrop. Gesell., No. 10 pp. 1–4.

'06 Zur Morphologie der Palma und Planta der Vorder-Inder und Ceyloner. Zeitschr. für Ethnol., 1906, pp. 656–706.

'06 Ueber das Leistenrelief der Hohlhand- und Fusssohlen-fläche der Halbaffen, Affen, und Menschenrassen. Ergebn. in der Anat. u. Entwick., Bd. XV., 1905, pp. 628–662. (An excellent review of the subject to date, with an exhaustive bibliography; this has been of much assistance in the present list.).

'12 Beobachtungsblatt und Anleitung zur Aufnahme von Hand- und Fussabdrücken. Korresp. bl. der deutschen anthropol. Gesell., Jahrg. XLIII., No. 5, 1912, pp. 33–36.

Schwalbe, G.

'05 Ueber Ballen, Linien, und Leisten der Hand. Strassb. medizin. Zeitung.

Seymour, Lee. (Three books, privately printed, and obtainable from the author, Los Angeles.) Finger-print Systems. Finger-print Classification. Finger-print Identification for Banks.

Varigny, M. H. de.

'91 Les empreintes digitales d'apres M. F. Galton. Revue Scientifique. T. XLVII., pp. 557–562.

Vucetich, Juan.

'01 Conferenzia sobre el sistema dactylosopicodada en la Bibliotheca publica de la Plata. La Plata, 1901.

'04 Dactyloscopia comparada. La Plata.

Welcker, H.

'97 Die Dauerhaftigkeit des Dessins der Riefchen und Fältchen der Hände. Arch. für Anthropol., Bd. XXV., pp. 29–32. (An interesting pioneer paper of four pages, with prints of the author's palm at 34 and again at 75 years. This is, perhaps, the first published print of a human palm. The print taken at the age of 34 must have been in 1858, and thus well antedates any work on the palm.)

Whipple, Inez L. (Mrs. H. H. Wilder).

'04 The Ventral Surface of the Mammalian *Chiridium*, with Especial Reference to the Condition Found in Man. Zeitschr. für Morphol. u. Anthropol., Bd. VII., pp. 261–368. (This is the fundamental paper on the comparative morphology of the ridge patterns of palms and soles, and includes the study of the relief of these surfaces in all mammals, and the growth of the ridges surfaces, as modified by this. This paper, with that of Schlaginhaufen, 1905, are of first importance in the scientific study of human friction-ridges. The book of Kidd, with the somewhat misleading title of "The Sense of Touch, etc.," 1907, is also of interest here.)

Wilder, Inez Whipple.

'08 Review of Kidd's "The Sense of Touch in Mammals and Birds, etc." Science, N. S., Vol. XXVII., pp. 582–585.

Wilder, H. H.

'97 On the Disposition of the Epidermic Folds upon the Palms and Soles of Primates. Anat. Anz., Bd. XIII., pp. 250–256.

'02 Scientific Palmistry. Pop. Sci. Monthly, Nov., 1902. (In this the four main lines were first used, but designated as lines 1–4, and described only as they limited the areas between them, upon which the attention was mainly directed.)

'02 Palms and Soles. Amer. Journ. Anat., Vol. I., pp. 423–441. (As in the previous paper, the description of individual palms and soles was based upon the areas rather than the main lines. The close similarity in the configuration of ridges in identical twins was also shown by two sets.)

'03 Palm and Sole Impressions and their Use for Purposes of Personal Identification. Pop. Sci. Monthly, Sep., 1903, pp. 385–410. (Here is shown for the first time the formulation of an individual palm by the course of the four main lines, which are designated as *A*, *B*, *C*, and *D*.)

'04 Racial Differences in Palm and Sole Configuration. Amer. Anthropol., Vol. VI., pp. 244–293. (Gives condition, with comparison, of the palmar and plantar configuration of Maya Indians, American Negroes, and American Whites. The Maya prints were collected by Dr. A. M. Tozzer.)

'04 Duplicate Twins and Double Monsters. Amer. Journ. Anat., Vol. III., pp. 387–472. (Continues the study of twins, with comparisons of their palms and soles.)

'08 Zur körperlichen Identität bei Zwillingen. Anat. Anz., Bd. XXXII., pp. 193–200. (Reports a case where an unusually complex palm pattern is reproduced, with but slight changes, on all four hands. The four feet are also duplicates.)

'12 Racial Differences in Palm and Sole Configuration: II. Palm and Sole Prints of Liberian Natives. Amer. Anthrop., Vol. XV., pp. 189–207. (This work is based upon 100 sets of prints, hands and feet, of Liberian soldiers, collected by Prof. Frederick Starr.)

Windt, Camillo.

'03 Ueber Dactyloscopie. Arch. für Kriminalanthrop., Bd. XII., pp. 101–123.

Windt u. Kodiczec.

'04 Dactyloscopie. Vienna.

Yvert, A.

'05 Identificación por las impressiones digitopalmares (La Dactiloscopie). Tésis pres. en la Univ. Lyon. Publ. La Plata.

Bibliography of Polyembryony in the Armadillo; Chronologically Arranged.

Fernandez, M.

'09 Beiträge zur Embryologie der Gürteltiere. Zur Keimblätterinversion und spezifischen Polyembryonie der Mulita (*Tatusia hybrida*). Morphol. Jahrb., Bd. XXXIX., pp. 302–333.

Newman, H. H. and Patterson, H. H.

'09 A Case of Normal Identical Quadruplets in the Nine-Banded Armadillo, and Its Bearing on the Problems of Identical Twins and Sex Determination. Biol. Bull., Vol. 17, pp. 181–187.

'10 Development of the Nine-banded Armadillo from the Primitive Streak Stage to Birth; with Especial Reference to the Question of Specific polyembryony. Journ. of Morphol., Vol. 21, pp. 359–423.

'11 The Limits of Hereditary Control in Armadillo Quadruplets: a Study of Blastogenic Variation. Journ. of Morphol., Vol. 22, pp. 855–926.

Patterson, J. T.

'12 A Preliminary Report on the Demonstration of Polyembryonic Development of the Armadillo. Anat. Anz., Bd. 41, pp. 369–381.

Newman, H. H.

'13 The Natural History of the Nine-banded Armadillo of Texas. Amer. Nat., Vol. 47, pp. 513–539.

'13 The Modes of Inheritance of Aggregates of Meristic (Integral) Variates in the Polyembryonic Offspring of the Nine-banded Armadillo. Journ. Exper. Zool., Vol. 15, pp. 145–192.

Patterson, J. T.

'13 Polyembryonic Development in *Tatusia novemcincta*. Journ. of Morphol., Vol. 24, pp. 559–662.

Newman, H. H.

'15 Heredity and Organic Symmetry in Armadillo Quadruplets. Biol. Bull., Vol. 29, pp. 1–32.

Vol. XXX. *April, 1916.* *No. 4*

BIOLOGICAL BULLETIN

THE INFLUENCE OF THE NUCLEUS ON THE BEHAVIOR OF AMŒBA.[1]

H. S. WILLIS.

INTRODUCTION.

OUTLINE.

PAGE.
Introduction 253
Material and Methods 255
Movement 256
  Normal specimens 256
  Fragments 256
Orientation in Light 263
  Normal Specimens 263
  Fragments 263
Rate of Locomotion 265
  Normal Specimens 265
  Fragments 265
Possible Causes of Differences in Behavior 267
  Size of Fragments 267
  Contractile Vacuole 267
  Position of Segments in Original Amœba 268
  Nucleus 268
The Influence of the Nucleus upon Attachment 268
Summary 269
Literature Cited 270

In the course of experimental work on *Amœba* in November, 1914, marked differences were observed in the behavior of the different parts of specimens cut in two. At the suggestion of Dr. S. O. Mast, an attempt has been made to ascertain whether these differences depend upon the nucleus and, if so, in what respects. I wish here to acknowledge my great indebtedness to Dr. Mast for his helpful and constructive suggestions and for his kindness in supervising both the experimental work and the writing of this paper.

---

[1] From the Zoölogical laboratory of the Johns Hopkins University.

Gruber (1912) found that parts of *Amœba proteus* containing a nucleus behave very much like normal specimens, but that parts without a nucleus behave very differently; yet, in spite of this, he held that the nucleus in general has no influence upon protoplasmic movement. Hofer (1890) in observations on the same species, obtained results similar to those of Gruber. He asserts (p. 118) that movements in the parts with the nucleus are similar to those in normal specimens, as are also those in the other parts for a period of 15–30 minutes after division, but that later the movements in the parts without a nucleus differ from those in normal specimens in rate of locomotion, in regularity of movement, and in the number and length of pseudopods.

Hofer holds that these differences might be considered to be due either to the injury received during the operation or to the influence of the nucleus. But he maintains that, since any injury sustained from the operation affects both fragments alike, the operation could not be considered the cause of the observed difference in behavior. He consequently concludes that the real cause is to be found in the nucleus. He holds, however, that the influence of the nucleus may be conceived to be direct or indirect; that is, that behavior may be due to an impairing of the elementary functions (such as digestion, respiration, and excretion) controlled by the nucleus. But Hofer found that the process of digestion in the absence of the nucleus continues for several days after division; that respiration takes place in the absence of the nucleus; and that excretory functions in enucleated segments continue till death. He therefore comes to the conclusion that the nucleus secretes a chemical substance and that behavior is controlled through this. He thinks a certain amount of this substance is stored up in the different parts of the protoplasm, and that the normal movement of the enucleated segments for 15–30 minutes after the operation is due to the influence of the substance thus stored. Hofer maintains that, since movement occurs in parts without a nucleus, cytoplasm has the power of movement; but since the movement in these parts is more irregular and haphazard than it is in nucleated parts, he holds that the nucleus must have a regulatory function. In other words, he thinks the nucleus serves as a regulatory "centrum" for behavior.

Verworn (1909) agrees with Hofer in holding that there is a difference in the behavior of parts of *Amœba* with and parts without a nucleus, but does not agree with him in the conclusion that the nucleus exerts a direct influence on the movement; that is, he does not think that the nucleus is a regulatory "centrum" for movement.

Judging from the results of my experiments, it is clear that there are distinct differences in the behavior of parts of *Amœba* which contain and parts which do not contain a nucleus. Differences in such parts have been observed in the character of movement, in the accuracy of orientation in light and in the rate of locomotion. It is clear, also, that these differences are traceable to the nucleus.

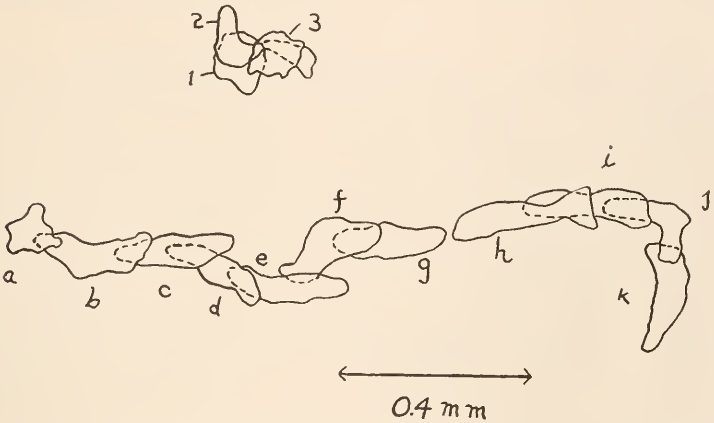


Fig. 1. Camera sketches showing changes in form and the characteristic movements in the nucleated and enucleated parts of an amœba immediately after being cut in two. The arrows indicate the direction of movement. *1–3*, enucleated part; *a–k*, nucleated part; *mm*, projected scale. The sketches of the enucleated part were made at five-minute intervals; those of the nucleated part at one minute intervals. Note that the nucleated part progressed regularly in a given direction, and that the enucleated part changed its form and position only slightly.

Material and Methods.

The specimens used for the most part in this work appeared in a battery jar containing an old paramecium culture. They corresponded to descriptions of *Amœba proteus* and were rather sensitive to light. Nucleated and enucleated parts were obtained by cutting specimens in two with finely drawn glass rods.

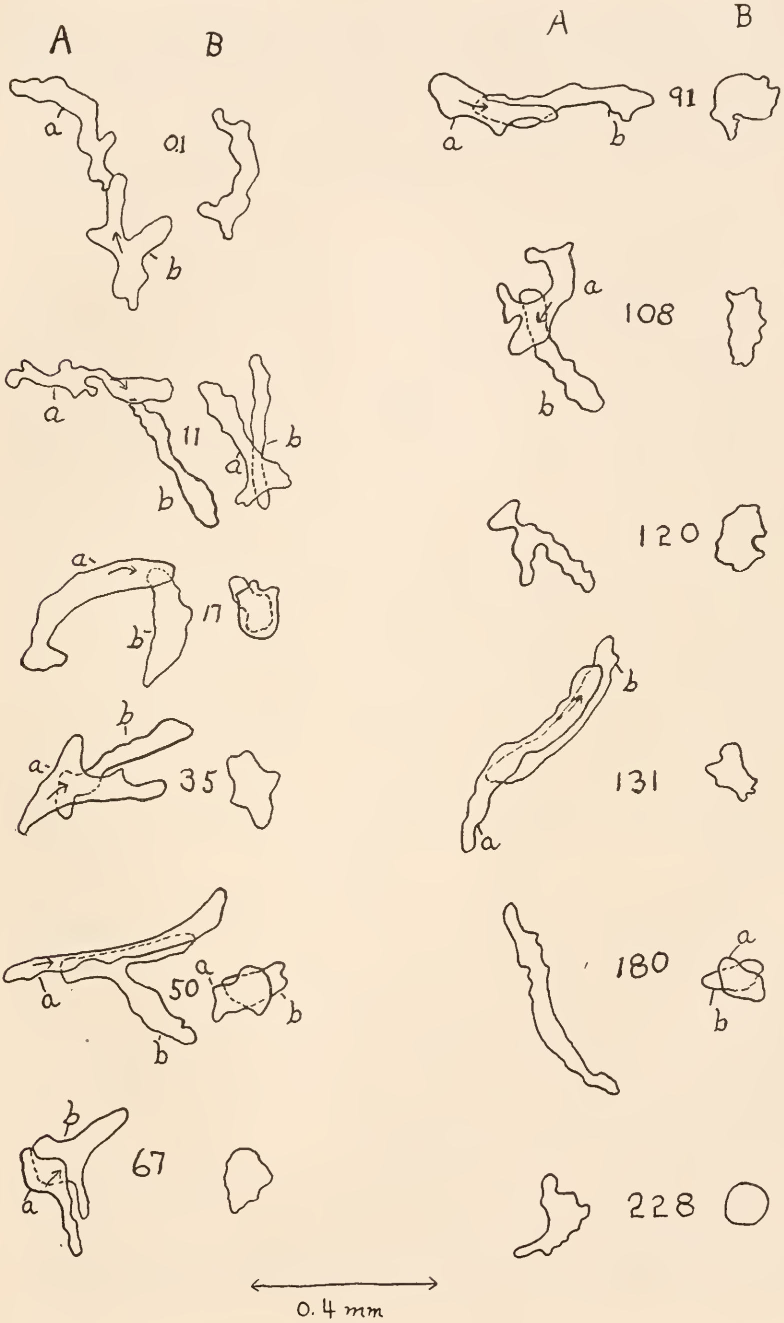
The cutting was done under a binocular. The parts of each individual were usually enclosed under a cover-glass by means of a ridge of vaseline so applied along the edge as to support the cover-glass and prevent drying. Thus the parts had entire freedom of movement and both were continuously subjected to the same environment; and, under these conditions, they were studied, observations being made both with a compound microscope and a binocular. Under the sealed cover-glasses the divided amœbæ did remarkably well, the nucleated parts living on an average of approximately ten days, *i. e.*, practically as long as normal specimens under the same conditions, and the enucleated parts about half as long. In the following descriptions, the two parts will frequently be designated fragments or segments.

MOVEMENT.

Normal Specimens.—In the process of locomotion in normal individuals of the species studied, pseudopods usually appear alternately on the two sides of the organism near the anterior end. A pseudopod appears, for instance, on the right, elongates and enlarges by the flow of protoplasm into it until it constitutes the main portion of the animal; then from this there is formed a new pseudopod on the left side. This, in turn, elongates and enlarges, after which a new pseudopod again forms on the right side, etc. Thus the organism takes a zigzag course.

Fragments.—In general it was found that the movement in fragments containing a nucleus is substantially like the movement exhibited in normal specimens, and, in some instances, for a period of 10–20 minutes after division, it was found to be similar in enucleated parts. Usually, however, the movement is strikingly different in such parts, it being slow and irregular, and frequently accompanied by contractions. The pseudopods, all

FIG. 2. Series of camera sketches illustrating the difference in the movement of nucleated and enucleated segments of *Amœba*. The former is shown in columns A, the latter in columns B. The numerals between the columns indicate approximately the intervals of time, in hours, between the cutting of the amœba and the production of the adjoining sketches in the two columns. The two sketches, *a* and *b*, in every case were made about one minute apart. They show the changes in position and in form of the segments during this time. Whenever there is but one sketch opposite the numerals, it indicates that there was no change in the organism. *a*, first position; *b*, second position, arrows, direction of movement, *mm*, projected scale.


A
B
A
B
a
0.1
b
91
a
b
108
a
b
a
11
b
a
b
120
a
17
b
b
131
a
b
a
35
a
50
b
b
180
a
b
b
a
67
228
0.4 mm

of which are usually small, are not formed alternately as they are in the parts containing the nucleus. Neither do they always occur near the anterior end. These differences in movement in the two segments are illustrated in Figs. 1 and 2.

The nucleated part represented in Fig. 1 formed pseudopods within one minute after division. At three minutes it was attached to the substratum and exhibited the coördinated movement characteristic of a normal individual (Fig. 1, *a*, *b*, *c*, etc.). This fragment was observed every three to five minutes for an hour, and the movement was found to be essentially the same throughout the entire period. The enucleated part of this individual on the contrary, became globular immediately and remained so for approximately five minutes during which time a number of small short pseudopods were directed outwards in all directions from the body. These became fewer and larger, and the entire body elongated in the direction of one of the larger pseudopods. An attachment of the body to the substratum was then formed and regular movement followed for about one minute. There was no subsequent locomotion or "streaming movement"; the organism, however, changed its shape by frequent contractions of the cytoplasm. These changes continued for an hour when the experiment was brought to a close (Fig. 1, 1–3). Throughout the entire period, the movement in the enucleated part was very much slower than that in the nucleated part.

Essentially all of these characteristic differences in the movements of the two fragments in question are further elucidated in the sketches reproduced in Fig. 2. By referring to this figure it will be seen that there was considerable locomotion in the nucleated part and that pseudopods were formed more or less regularly on the opposite sides of the segment; while in the enucleated segment there was extremely little locomotion and pseudopods were formed irregularly.

Figs. 1 and 2 are typical illustrations of the behavior observed in all of the specimens studied—forty in number. The movement in all nucleated parts of these specimens was like that in normal specimens and the movement in all enucleated parts was quite different from that in normal specimens. The behavior of 17 pairs of these parts taken at random is briefly summarized in Table I. By referring to this table, in which observations on locomotion are recorded for 13 of the 17 individuals, it will be seen that locomotion occurred in 13 of the nucleated parts, while there was no locomotion in the enucleated parts. Other matters in this table will be considered later.

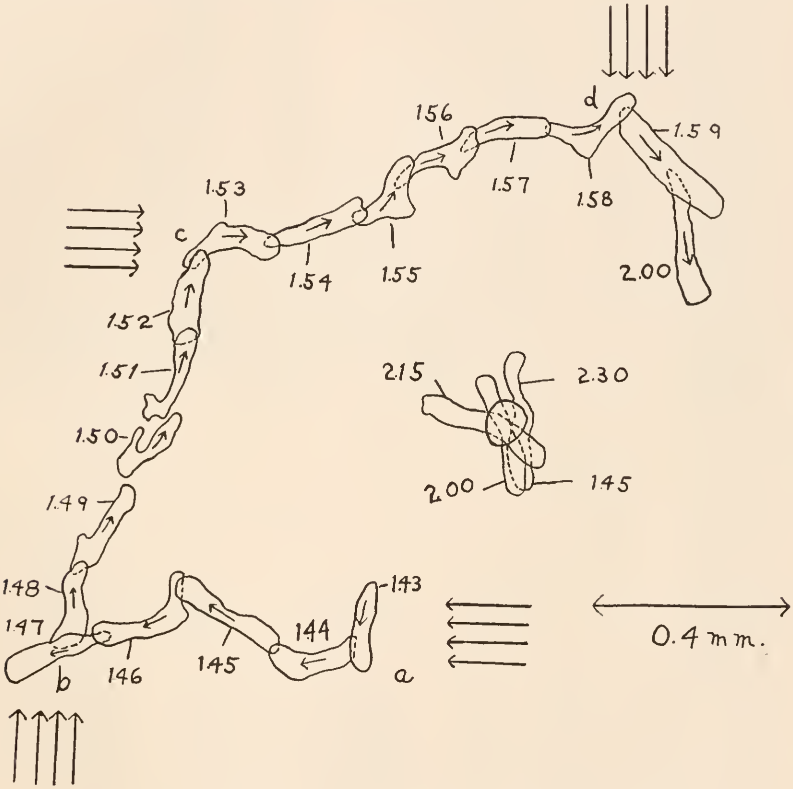


FIG. 3. A series of camera sketches showing the reactions of a nucleated and an enucleated part of an amœba in a horizontal beam of light. *a–d*, nucleated part; 1.45–2.30 enucleated part; large arrows, beam of light; small arrows, direction of movement; 1.43–2.00, time at which sketches of nucleated part were made; 1.45–2.30, time at which sketches of enucleated part were made; *mm.*, projected scale. Note that the nucleated segment oriented fairly definitely; at *a* (1.43) the rays of light were at right angles to the moving segment; at 1.44 the segment had turned and become directed from the source of light. A similar response occurred at *b*, *c*, and *d* after the direction of the rays of light had been changed in each case. Note also that the enucleated part did not orient in the light. Both parts were continuously in the same field and were subjected to the same changes in illumination.

TABLE I.

Table showing the relation between the reactions of fragments of *Amœba* and the presence or absence of the nucleus; the position the fragments occupied in the intact specimens; the size of the fragments; the length of time the various pairs of fragments were studied, and the attachment of the parts to the substratum. Note that the nucleated parts, regardless of size or position, remained attached to the substratum and responded essentially like normal specimens, and that the enucleated parts rarely, if ever, became attached and did not respond like normal specimens.

Designation of the Individuals Cut into Two Parts.	Contents of Parts.	Position Occupied by the Two Parts in Intact Amœba.	Relative Size of Parts.	Length of Time Studied (in Hours).	Relation of Parts to Substratum.	Behavior.
1	Nucleus.	Anterior end.	Slightly larger.	¾	Part attached 1 min. after division.	Locomotion.
	No nucleus.	Posterior end.			Attached one min. after division. Attachment lost after 5 mins. Regained and lost in first 20 min. after division.	Slight motion.
2	Nucleus.	Anterior end.	Larger.	½	Attached after 2 min.	Locomotion.
	No nucleus.	Posterior end.			Did not attach.	Became spherical.
3	Nucleus.	Anterior end.	Equal.	1/6	Attached soon after division.	Locomotion.
	No nucleus.	Posterior end.			Attached slightly.	No locomotion.
4	Nucleus.	Posterior end.	Equal.	1/5	Attached 2 min. after division.	
	No nucleus.	Anterior end.			Did not attach.	
5	Nucleus.	Anterior end.	Equal.	¼	Attached 2 min. after division and remained so.	
	No nucleus.	Posterior end.			Did not attach.	
6	Nucleus.	Posterior end.	Equal.	1⅓	Attached.	Movement normal.
	No nucleus.	Anterior end.	Equal.		Attached 30 min. after division. Attachment broken by gentle blowing on the water. Did not attach again.	Irregular motion.
7	Nucleus.	Anterior end.	Larger.	1	Attached 7 min. after division and remained so.	Movement normal.
	No nucleus.	Posterior end.			Did not attach.	Became spherical.
8	Nucleus.	Anterior end.	⅓ larger.	1	Attached 3 min. after division and remained so.	Movement normal.
	No nucleus.	Posterior end.			Attached slightly 25 min. after division; lost at 30 min. No further attachment.	Pseudopods irregularly formed.

TABLE I.—*Continued.*

Designation of the Individuals Cut Into Two Parts.	Contents of Parts.	Position Occupied by the Two Parts in Intact Amœba.	Relative Size of Parts.	Length of Time Studied (in Hours).	Relation of Parts to Substratum.	Behavior.
9	Nucleus.	Posterior end.	Equal.	1	Attached 2 min. after cutting. Attachment broken 5 min. later. Attached again in 1 min. and remained so.	Locomotion.
	No nucleus.	Anterior end.			Attached 2 min. after division. Unattached 5 min. later. Attached at 20 min. after division. Unattached at 25 min. and remained so.	Irregular motion.
10	Nucleus.	Anterior end.	Smaller.	1	Did not attach for an hour.	
	No nucleus.	Posterior end.			Did not attach for an hour.	
11	Nucleus.	Posterior end.	Equal.	1½	Remained attached after first 10 min.	
	No nucleus.	Anterior end.			Unattached for 1 hour when it slightly attached. Remained attached 15 min. No further attachment.	
12	Nucleus.	Posterior end.	Larger.	2½	Attached 5 min. after cutting. Attachment broken 3 min. later. Attached again 10 min. after division, and remained so.	Locomotion.
	No nucleus.	Anterior end.			Attached 15 min. after division. Attachment broken 3 min. later. It did not attach again.	
13	Nucleus.	Anterior end.	Equal.	3	Attached 5 min. after division. Attachment broken 3 min. later. Formed again after 2 min. and remained so.	Locomotion.
	No nucleus.	Posterior end.			Attached 5 min. after division. Attachment broken 3 min. later; then formed again after 20 min. This was lost 2 min. later. No further attachment.	Irregular motion.

TABLE I.—*Continued.*

Designation of the Individuals Cut into Two Parts.	Contents of Parts.	Position Occupied by the Two Parts in Intact Amœba.	Relative Size of Parts.	Length of Time Studied (in Hours).	Relation of Parts to Substratum.	Behavior.
14	Nucleus.	Anterior end.	⅓ larger.	3	Attached 4 min. after cutting and remained so.	Locomotion.
	No nucleus.	Posterior end.			Attached 1 min. after division. Unattached at 5 min. after. 15 min. later attached again for several min. No subsequent attachment.	Much contraction.
15	Nucleus.	Posterior end.	Smaller.	52	Attached 5 min. after division. Attachment was noticed at many observations during the 52 hours.	Locomotion.
	No nucleus.	Anterior end.			Observed at intervals of 5 min. each for a few hours, then observed every few hours. No attachment.	Contractions.
16	Nucleus.	Posterior end.	Equal.	72	Attached 10 min. after division and remained so.	Locomotion.
	No nucleus.	Anterior end.			No attachment.	No locomotion.
17	Nucleus.	Posterior end.	Equal.	72	Remained attached after 1 min. following division.	Locomotion.
	No nucleus.	Anterior end.			Attached 5 min. after division. Remained attached for ½ hour. No further attachment.	Contractions.

It has been thus shown that there is a difference in the general movements of the segments containing and those not containing a nucleus. Attention is now directed to the reactions to light in such segments, especially the reactions resulting in orientation.

Orientation in Light.

Normal Specimens.—It is well known that certain species of *Amœba* when placed in a horizontal beam of light, usually turn until they are directed from the source of light and then continue in a fairly direct course; that is, they orient and are negative. If now the position of the source of stimulation is changed so as to illuminate the organisms from the side, they again turn until they are directed from the light, that is, they reorient. In the form under consideration the reactions to light are marked and orientation is fairly precise.

Fragments.—Fragments containing a nucleus present precisely the same kind of reaction as do normal specimens when subjected to similar light conditions. These parts react readily to light; the movement is rapid; and the process of orientation is very much like that found in intact specimens. On the other hand, enucleated parts usually give no evidence whatever of orientation. Experiments were made on 25 pairs of segments. In 21 of these the nucleated parts oriented approximately as precisely as normal individuals, but in the enucleated parts no indication of orientation whatever was observed except in three instances, and in these there was only a mere suggestion of orientation. This will be described in more detail later.

Typical of the reactions in the 25 experiments mentioned above are the responses to light of the parts shown in Fig. 3. The reactions here represented, however, are somewhat more exact and definite than those observed in some of the other segments. In this case the enucleated fragment was the larger of the two and contained the contractile vacuole. Both segments were kept under the same cover-glass and were subjected to the same light conditions. A 165-C. P. tungsten lamp was placed 13 cm. distant from the reacting segment, and a piece of colorless glass absorbed the heat waves from the lamp. A compound microscope and a camera lucida were used. The temperature at the microscope was 29.5° C. The results obtained are in part recorded in Fig. 3. It may be observed from this figure that the nucleated part moved from the light and that with each change in the direction of the rays of light, there was a corresponding change in the direction of the movement of the segment, while on the other hand, there was no indication of orientation in the enucleated fragment. The two parts were subjected to precisely the same conditions throughout the entire experiment.

As previously stated, there was usually no indication whatever of orientation in enucleated segments, and it is questionable whether any of these parts actually oriented, yet, in a number of cases, slight movement from the light occurred, and in three cases there was a change in the direction of movement with a change in the direction of the light. This movement from the light may have been a reaction to the light in the form of orientation or it may have been merely an accidental movement made without regard to the light. The facts regarding the three cases are these. One of the segments, while moving at right angles to the light, formed a pseudopod on the shaded side, and movement occurred in the direction of the pseudopod. With a change in the direction of the light, a short pseudopod was formed again on the shaded side, but at this point the fragment assumed a globular shape. In another segment movement occurred for several seconds at right angles to a horizontal beam of light, then the direction was changed, and the fragment moved from the source of stimulation. Upon a change in the direction of the light, this fragment changed the direction of its course and again moved from the light for approximately one minute and then became globular. In the third enucleated segment substantially the same reaction was observed. If these fragments actually oriented in response to the light, the process of orientation in them was essentially different from that in nucleated fragments.

There is, therefore, a difference in nucleated and enucleated parts of *Amœba* in their response to light as well as in the character of their movements; but there is also a difference in the rate of locomotion as will be demonstrated presently.

RATE OF LOCOMOTION.

Normal Specimens.—The rate of locomotion varies greatly in different individuals. Some specimens move nearly twice as fast as others. Quite a number of individuals were observed to move rather consistently at 0.27–0.3 mm. per minute; others moved at an average rate of 0.12–0.15 mm. per minute. There is a fairly definite relation between the size of the moving organism and the rate of movement. An animal travels a distance that is approximately two thirds the length of its body in one minute. The actual distance traveled by a large animal is greater for a given length of time than the actual distance traveled by a small animal for the same length of time under the same conditions. This is clearly demonstrated by the results recorded in Table II. By referring to this table it will be seen that the ratio of the distance traveled per minute to the length of the body of the specimen is approximately the same in all cases.

TABLE II.

Relation between size of *Amœba* and rate of locomotion. The average rate of locomotion was computed from five readings extending one minute each. The average length was computed from five measurements of the moving organism at intervals of one minute each. The rate was measured by projecting the moving organism on a scale with a camera lucida.

Maximum Length of Specimens Observed.	Average Distance Traveled per Minute.	Ratio of Distance Traveled per Minute to Length of Body.
0.36 mm.	0.24 mm.	0.66
0.34 "	0.20 "	0.59
0.35 "	0.23 "	0.65
0.37 "	0.24 "	0.65
0.21 "	0.15 "	0.71
0.26 "	0.17 "	0.64
0.20 "	0.14 "	0.72
0.26 "	0.17 "	0.64

Fragments.—In the work on the rate of locomotion in fragments, the two parts were on an average approximately the same size. Observations were made on each pair of segments at about the same time. Both were kept under the same cover-glass and in the same environment. It was found that the rate of movement in nucleated fragments, like that in normal specimens, bears a definite relation to the size of the body. Large nucleated segments usually travel greater distances in a given length of time than do small ones. This is shown in the results recorded in Table III. In this table are given the lengths of four intact specimens together with the rate of locomotion per minute, and the length of body, and the rate of locomotion in the nucleated and enucleated parts cut from the four intact specimens.

TABLE III.

Relation between rate of locomotion and length of body in four intact specimens of *Amœba* and in the nucleated and enucleated parts of these four specimens.

Normal Specimen.		Nucleated Part.		Enucleated Part.	
Average Length of Body.	Rate of Locomotion per Minute.	Average Length of Body.	Rate of Locomotion per Minute.	Average Length of Body.	Rate of Locomotion per Minute.
.376 mm.	.244 mm.	.275 mm.	.150 mm.	globular	negligible
.355 "	.230 "	.210 "	.150 "		
.340 "	.200 "	.220 "	.140 "	.230 mm.	?
.360 "	.240 "	.200 "	.128 "	.230 "	.074 mm.

The table shows that the larger intact specimens and the larger nucleated parts travel greater distances per minute than do the smaller ones. It also shows that locomotion in the enucleated parts is very much slower than it is in the nucleated parts. This is, however, more clearly shown in Table IV in which the rate of locomotion in nucleated and enucleated segments of the same individual is compared. Each number in the two columns of this table represents the average rate of

TABLE IV.

A comparison of the rate of locomotion in millimeters per minute in the nucleated and the enucleated parts of ten different individuals.

Nucleated Parts.	Enucleated Parts.
0.15	0.002
0.13	0.074
0.13	0.010
0.18	0.020
0.13	0.008
0.15	0.110
0.16	0.000
0.19	0.006
0.17	0.006
0.13	0.004

locomotion for five trials, each continuing for one minute. The figures in the left column show the rate of locomotion in the nucleated parts; those figures immediately opposite, in the other column, show the rate of locomotion in enucleated parts. For instance, the nucleated segment of the individual first cut moved in five trials at an average rate of 0.15 mm. per minute, and the enucleated part cut from the same individual moved at an average rate of 0.002 mm. per minute. From the table one thing is strikingly evident: movement in nucleated parts is always much more rapid than that in enucleated parts; in no case does the rate of locomotion in parts without a nucleus equal that in parts with a nucleus.

Possible Causes of Differences in Behavior.

From the experiments cited above, it is evident that there are differences in the behavior of parts of *Amœba* with a nucleus and parts without a nucleus; differences in the character of movement, in orientation, and in the rate of locomotion. Now, to what may these differences be attributed? The two parts differed in size, in their position in the amœba cut to produce them, in the possession of a contractile vacuole and a nucleus. The difference observed in their behavior must be ascribed to some of these differences in structure.

Size.—In this work nearly 125 amœbæ were cut, each into two parts, one of which contained a nucleus. In approximately one half of these cases, the part without a nucleus was as large as or larger than the part with a nucleus. Records of the two parts of 13 of these amœbæ, taken at random, may be found in Table I. Both parts in all cases were kept under the same cover-glass and were subjected to the same or similar conditions. All of the nucleated parts behaved like normal specimens, but none of the enucleated parts, regardless of the relative size of the two parts. It appears, therefore, that differences in the size of parts do not determine the differences in behavior.

Contractile Vacuole.—The contractile vacuole was present in the nucleated parts of the 125 specimens experimented upon about as often as it was in the enucleated parts. There was no observable difference in behavior associated with the presence or absence of the vacuole. Enucleated parts with the vacuole apeared to behave in every way similarly to enucleated parts without a vacuole. The same is true for nucleated parts. The vacuole, therefore, cannot be reckoned as a determining factor in the behavior of the fragments.

Position Occupied by Fragments in the Intact Amœba.—When an amœba is cut into two parts, the part containing the nucleus may be from what was the anterior end or the posterior end of the original specimen. The behavior was carefully studied in 25 parts, 13 from the anterior and 12 from the posterior end of the intact individual. The results obtained are recorded in Table I. No evidence was obtained indicating that the reaction of the parts depends upon their location in the specimens from which they were taken. The nucleated parts from the anterior end responded precisely like those from the posterior end. Obviously, then, the position in the intact specimen is not a determining factor in the behavior of fragments.

The Nucleus.—We have demonstrated that parts of *Amœba* which contain a nucleus behave essentially like normal specimens, while those which do not contain a nucleus behave quite differently, and that this difference in the behavior of the parts is dependent upon neither their relative size, the presence of the contractile vacuole, nor the location of the parts in the animals which were cut to produce them. It must, therefore, in some way, be related to the nucleus. This will be considered in the following paragraphs.

The Influence of the Nucleus upon Attachment.

Dellenger (1906) made it clear that attachment always accompanies and is essential to efficient locomotion in *Amœba* and *Difflugia*. Twenty-five of my experiments on fragments of *Amœba*, in which observations were made on attachment, demonstrate quite clearly that nucleated fragments are usually continuously attached to the substratum, and that enucleated fragments are rarely attached. In these experiments some specimens were observed for only ten minutes; others for a very much longer period of time, the maximum being 72 hours. Records of attachment or unattachment in all were made at definite intervals. The following detailed description of the results obtained in the study of one pair of segments taken from the same specimen is typical of all. In this case the parts were approximately equal in size and the nucleated part was cut from the anterior end of the amœba.

At five minutes after division both fragments were attached and moving. Three minutes later the attachment in both was broken by the sliding of a small glass rod under them. The nucleated part began immediately to send out pseudopods aimlessly. This continued for two minutes; then the segment attached and remained so for three hours—when the experiment was brought to a close. This segment was attached continuously to the substratum; it appeared to be attached usually at three different points and the behavior appeared to be normal in every respect. The enucleated segment was momentarily attached at several different times, and each time the attachment was so weak that it could not resist even the slightest jar given the table on which the experiment was made. At no time was this segment continuously attached longer than two minutes and it was never attached at more than one point at a time. There was a slow streaming motion in the protoplasm but no locomotion at all except for a short time during its first attachment and then it was very slight. The results obtained in all of the observations on the attachment of segments of *Amœba* are briefly summarized in Table I. By referring to this table it will be seen that attachment of nucleated parts to the substratum is *continuous;* that attachment of enucleated parts is *intermittent, slight* and of *short duration.*

These facts seem to indicate that the nucleus directly influences the attachment of protoplasm to the substratum and thus influences behavior.

SUMMARY.

1. *Amœba proteus* moves regularly and smoothly by alternate formation of pseudopods on the two sides of the organism. Locomotion in segments of *Amœba* with a nucleus is of the same general character. Movement in segments without a nucleus, however, is irregular, jerky, very much slower than that in nucleated parts, and the pseudopods are not ordinarily formed regularly or alternately.

2. In a horizontal beam of light, normal specimens direct

their locomotion from the source of stimulation, *i. e.*, they orient and are negative. Parts containing a nucleus respond in the same manner; those without a nucleus, however, do not orient.

3. The rate of locomotion varies greatly in different individuals. Large specimens move more rapidly than small specimens, the rate of locomotion bearing a fairly definite ratio to the size of the specimen. The rate of locomotion in nucleated segments bears essentially the same ratio to their size as it does in normal individuals. Segments without a nucleus show very little locomotion and this is always relatively very slow and irregular.

4. The size of the parts, the contractile vacuole, and the position which the parts occupied in the intact specimens before division seem to be in no way responsible for differences in the behavior of nucleated and enucleated parts of *Amœba*.

5. The only other known respect—aside from those mentioned—in which the two parts differ concerns the nucleus. Consequently the differences in the behavior of these parts are, in all probability, in some way related to the nucleus.

6. The regulatory influence of the nucleus on behavior in *Amœba* seems to be brought about by some sort of an influence upon the attachment of the organism to the substratum.

LITERATURE CITED.

Dellenger, O. P.
'06 Locomotion of Amœbæ and Allied Forms. Jour. Exp. Zool., Vol. 3, pp. 337–358.
Gruber, K.
'12 Biologische und experimentelle Untersuchungen an Amœba proteus. Arch. f. Protistenk., Bd. 25, S. 316–376.
Hofer, Bruno.
'90 Einfluss des Kerns auf das Protoplasmas, Jena. Zeit. f. Nat., Bd. 24, S. 105–175.
Jennings, H. S.
'04 Contributions to the Study of the Behavior of Lower Organisms. Carnegie Inst. of Washington, Pub. No. 16, 256 pp.
'06 Behavior of Lower Organisms. New York. 366 pp.
Mast, S. O.
'11 Light and the Behavior of Organisms. New York. 410 pp.
'10 Reactions in Amœba to Light. Jour. Exp. Zoöl., Vol. 9, pp. 265–277.
Štolc, A.
'10 Über kernlose Individuen und kernlose Teile von Amœba proteus. Arch. f. Entom., Bd. 29, S. 152–168.
Verworn, Max.
'09 Allgemeine Physiologie, 700 pp.

NOTES ON SUPERFETATION AND DEFERRED FERTILIZATION AMONG MICE.

F. B. SUMNER,

Scripps Institution for Biological Research, La Jolla, Calif.

The phenomenon of superfetation, or the occurrence of a second conception in a mammal already pregnant, has been reported and discussed by various writers since the time of Aristotle. The latter[1] refers specifically, in this connection, only to man and the hare. Modern writers[2] have described occasional instances for several animals (man, cat, rat, sheep). On the other hand, this phenomenon appears to have been generally regarded as a biological curiosity, while its very existence has been questioned by some,[3] the facts observed being explained on other grounds. Apparently, it has been very rarely observed in the rat, for Miss King records only two cases among the more than 700 normal broods born in her stock.[4] For mice, I have thus far found no published records of supernumerary broods, though two well-known zoölogists, who have reared varieties of the house mouse in large numbers, assure me that they have encountered cases of this sort. In the absence of exact records, I am unable, however, to report these instances in detail.

In the course of several years' experience in breeding white mice, the present writer has occasionally noted the birth of a second litter, in cases where an earlier litter had been kept with the mother for some time after weaning. This was at first attributed to the precocious sexual development of the earlier male offspring, one of which was held to be the father of the later brood. In one case of which I have record (Table II., No. 1), this may well have been the true explanation, since the second conception must have occurred when the three males which constituted the first brood were about 42 days old. That male, and even female, white mice may become sexually mature at this age I am certain from independent evidence. I have record of at least one case in which a female, mated to a male of the same brood, became pregnant at an age of about 6 weeks (between 38 and 44 days).

---

[1] See bibliographic references.

[2] Marshall, 1910; King, 1913 (also papers cited by the latter).

[3] Schultze, 1866; Godlewski, 1914.

[4] I do not here include those cases (known as superfecundation), in which members of the same litter, derived from the same period of ovulation, are born one or two days apart. I have myself observed a few cases of this phenomenon, but shall not discuss them here.

In one instance, however (Table I., no. 6) such an explanation was not applicable, owing to the extreme youth of the first litter, at the time of the second conception. On March 11, 1909, a female gave birth to a brood of two (1 ♂, 1 ♀). On April 8, or 28 days later, a second brood of seven was born. Since the period of gestation of this animal is normally about 20 days,[1] the mice of the first litter were scarcely more than a week old when the second lot began their development. It is needless to point out that a blind and helpless young male of this age could not have played the part of a father. Furthermore, the female had been separated from all other adults of either sex, since the day of the birth of the first brood of young, 28 days previously. Under these circumstances, to be sure, the possibility is not excluded that the second litter resulted from a copulation occurring immediately after the birth of the first. In this event, the only anomaly would be the deferred birth of the later brood of young.

Unfortunately, no further notes on this subject were made during my experiments on white mice. It is my impression that other instances similar to the foregoing were observed, but I find record of only these two cases.

It is the chief object of the present paper to furnish data relating to superfetation and deferred fertilization which have been recorded incidentally during the past two years, in the course of rearing wild mice of the genus *Peromyscus*. Unfortunately, my data are in some cases somewhat equivocal, since the experiments were not made with a view to studying these particular problems. But definite answers can none the less be given to certain questions, even if not to others.

---

[1] My own observations confirm those of Daniel (1910) on this point.

My stock comprises four geographical races of *Peromyscus maniculatus*, viz., *rubidus*, *sonoriensis*, and two quite distinguishable races within the assemblage generally known as "*gambeli*."

In breeding these mice, it is my general practice to place one male with three to five females. Each male is left with the respective group of females for a varying period, which ordinarily does not exceed 20 days. Record is in every case kept of the day when the male is admitted, and of the day when he is removed. Each female, when obviously pregnant, is isolated in a separate cage. The young are ordinarily left with the mother until they are 6 to 8 weeks old. At this time, they are commonly separated according to sex, the males and females being thenceforth kept apart until they are mated, according to the requirements of the work. Occasionally, this separating is not done early enough, however, and matings between brother and sister, or between mother and son, occur. The youngest mice in which I have known fertilization to take place were both 42 days old (possibly a few days younger), the next youngest pair were 55 days old or less. Because of these precocious, and therefore, unexpected conceptions, the exact date of birth of the resulting young cannot always be recorded. They were sometimes several days old when first found. Now it is among very young parents that the phenomena to be discussed seem to be commonest, as will be pointed out presently. For this reason, it happens that in all but two among the eight cases comprised in my first table the father was still present in the cage, and had access to the mother up to the time of the isolation of the latter, when her brood was first discovered. This uncertainty as to the date of the last coition somewhat complicates the interpretation of these cases, though, as will be shown, the evidence, even here, for a deferred fertilization of the ova is not thereby affected.

Before considering the phenomena presented by the tables, it will be well to make inquiry as to the normal period of gestation in *Peromyscus*. Since I have been interested only incidentally in determining this period, I have in no case restricted the mating of the parents to a single observed copulation, as has Daniel (1910), nor have I determined the exact hour of the birth of the brood, as have Long and Mark (1911). I have recorded, however, the period during which the male had access to the female, and the date of discovery of the brood. During the times when frequent births were expected, the nests were examined daily. Hence a given birth must commonly have occurred between two known observations. It has been my invariable practice to refer the birth to the day when the brood was discovered,[1] except where circumstances were such as to make it probable that the young were born a day or two earlier. In such cases, this probability is indicated in the records.

Since, in a large series of experiments, it is probable that successful insemination would occur in some cases pretty soon after the males were introduced into the cages, it seems likely that the *minimum* interval between the introduction of a male and the birth of a brood represents the actual period of gestation, at least for the individuals in question. This minimum was found to be 22 days. In all but five cases, however, out of a total of 206 broods, for which I have accurate records, the figure was greater than this. As a control, I have determined, for each series, the *maximum* interval between the removal of the male and the discovery of a brood of young. This maximum figure is also a fraction over 22 days. In the single instance recorded, a brood was known to be born as late as some time during the 23d day after the last opportunity for copulation.

From these data, therefore, we know that the period of gestation of *Peromyscus* does not, in some cases at least, *exceed* 22 days. We also know that it may be *as much as* 22 days. Accordingly, I have provisionally adopted this figure as representing the normal term for the species which I am engaged in breeding. It is likely, however, that this term is subject to some variation.

Tables I. and II. comprise two different classes of cases. In the former, the interval between the birth of the first and second litters ranges from 13 to 39 days. In all of these cases the youth of the first brood of young, at the time of the second conception, precludes the possibility that a male of the former lot was the father of the second. Even when the interval was as great as 39 days, conception must have occurred when the first lot was

---

[1] Mice (at least white mice) are said by Long and Mark (1911) to be more commonly born in the early morning than at any other time of day.

Table I.

Cases in Which a Male of the First Litter Could Not Have Been the Father of the Second.

Case No.	Race.	Last Opportunity for Copulation.	Date of Birth of First Litter.	Number When First Counted.	Number Surviving Nearly or Quite to Maturity.	Interval Between First and Second Litters.	Date of Birth of Second Litter.	Number in Second Litter.
1	*gambeli* (LaJ.)	Aug. 12 (or before)	Aug. 12, 1915.	1	1 ♂	13 d. (±)	Aug. 25 (±)	3
2	*gambeli* (Berkeley)	July 30	July 30, 1915	2	none	26 d.	Aug. 25	3
3	*gambeli* (LaJ.)	Aug. 11	Aug. 11, 1915 (or before)	1	1 ♂	27 d. (±)	Sept. 7 (or before)	4
4	*gambeli* (LaJ.)	Aug. 11	Aug. 11, 1915 (or before)	1	1 ♀	27 d. (±)	Sept. 7 (or before)	5
5	*rubidus*	May 17	June 5, 1915.	2	2 ♀	28 d. (±)	July 3 (±)	3
6	*albino* (Mus)	March 11	March 11, 1909.	2	1 ♂, 1 ♀	28 d.	Apr. 8	7
7	*sonoriensis*	Aug. 12	Aug. 12, 1915 (or before)	3	3 ♂	37 d. (or more)	Sept. 18	4
8	F_1 hybrid (*sonoriensis* × *gambeli*)	Aug. 31	Aug. 28, 1915 (±)	3	2 ♂	39 d. (±)	Oct. 6 (±)	3

not over 17 days old, *i. e.*, when the young animals had just opened their eyes. At 4 and 6 days, the young are, of course, blind and helpless. Indeed, in two cases—were further proof needed—it will be noted that the first brood contained no males, while in still another, they had not yet been born.

In cases of the second class (Table II.), the first and second litters were born 63 to 81 days apart, *i. e.*, the second conception occurred when the first brood—which in every case contained a male—was 41 to 59 days old. In these cases, the possibility is by no means excluded that a member of the first litter served as the father of the second. As already stated, there is positive evidence, both in the white mouse and in *Peromyscus*, that fertilization may occur at an age of six weeks. On the other hand, such precocity is quite exceptional, so that this explanation of the cases in Table II. seems hardly more probable than that by which we must explain those in Table I., viz., fertilization by spermatozoa received from the earlier mate. In the absence of definite evidence, however, it would be idle to speculate regarding the relative probability of these two interpretations, and I shall, accordingly, exclude the cases in Table II. from most of the ensuing discussion. Nevertheless, it is worth pointing out—what is not indicated in the table—that the first births recorded in cases 3 and 4 of Table I. are the same as the second births recorded in cases 5 and 9 of Table II. If, therefore, these latter are likewise instances of deferred fertilization, we must assume for these two mothers *the delivery of three consecutive broods, after separation from their mates*. The possibility of such a surprising occurrence surely warrants more carefully controlled experiments in the future.

In the more familiar discussions of superfetation, it appears to be invariably assumed that the later fetuses owe their existence to coition occurring *during the first pregnancy*. This assumption is made by Aristotle, in the passages referred to. Marshall (1910, p. 159) says: "If ovulation takes place during pregnancy, and if, owing to the occurrence of coition the ova become fertilized, the phenomenon of superfoetation may take place—that is to say, foetuses of different ages may be present in the same uterus."

TABLE II.

CASES IN WHICH A MALE OF THE FIRST LITTER MAY HAVE BEEN THE FATHER OF THE SECOND.

Case No.	Race.	Last Opportunity for Copulation.	Date of Birth of First Litter.	Number When First Counted.	Number Surviving Nearly or Quite to Maturity.	Interval Between First and Second Litters.	Date of Birth of Second Litter.	Number in Second Litter.
1	*albino* (Mus)	?	Oct. 30, 1908	3	3 ♂	63 d. (±)	Jan. 1 (±)	?
2	*sonoriensis*	Aug. 16	Aug. 23, 1915	4	1 ♂, 3 ♀	71 d. (or less)	Nov. 2 (or before)	2
3	*sonoriensis*	Aug. 16	Aug. 22, 1915	4	2 ♂, 2 ♀	73 d.	Nov. 3	1
4	*gambeli* (LaJ.)	Aug. 16	Aug. 21, 1915	3	1 ♂	74 d.	Nov. 3	4
5	*gambeli* (LaJ.)	May 17	May 28, 1915	2	1 ♂, 1 ♀	75 d. (or less)	Aug. 11 (or before)	1
6	*sonoriensis*	?	June 1, 1914	3	1 ♂, 1 ♀	75 d.	Aug. 15	3
7	*rubidus*	May 17	May 24, 1915	2	1 ♂, 1 ♀	79 d. (or less)	Aug. 11 (or before)	3
8	*sonoriensis*	Aug. 16	Aug. 20, 1915	2	2 ♂	79 d. (or less)	Nov. 11 (or before)	4
9	*gambeli* (LaJ.)	May 17	May 22, 1915	4	1 ♂, 1 ♀	81 d. (or less)	Aug. 11 (or before)	1

Miss King (1913, p. 385) remarks that "superfœtation . . . is applied to cases in which ovulation, followed by copulation, occurred during pregnancy and led to the simultaneous development in the uterus of two sets of ova belonging to *different* periods of ovulation." In discussing the two instances observed by her in the rat she says: "The most plausible explanation for these cases seems to me to assume that the two ovaries acted independently, ovulation occurring in one ovary some little time before it took place in the other. If copulation followed each ovulation two sets of embryos would develop in the uterus simultaneously, and they would be born at different times, depending on the interval between the two periods of ovulation" (p. 390).

I believe, with the writers quoted, and in opposition to certain others, that these younger fetuses owe their origin to later periods of ovulation, which may even occur during gestation. I cannot see the necessity, however, for assuming an independent action of the two ovaries, as does Miss King. Furthermore, I regard it as highly improbable that some of the cases which I have recorded resulted from a copulation occurring at the time of the later ovulation, whether this last took place during pregnancy or after the birth of the first brood.

My own observations point, with considerable probability, to two facts: (1) *to a definite periodicity in ovulation, continuing in some cases throughout pregnancy; and* (2), *with even greater probability, to the retention by the spermatozoa of their fertilizing power for days or even weeks after reception into the uterus or fallopian tubes.*

In support of the first proposition I will point to the rather surprising numerical relations among the figures (Table I.) representing the intervals between the delivery of the two broods by the same mother. These eight numbers are all nearly or quite multiples of 13, viz:

1 × 13 (±)

2 × 13
2 × 13, (+ 1?)
2 × 13, (+ 1?)

2 × 13, (+ 2?)
2 × 13, (+ 2)

3 × 13, (− 2?)
3 × 13, (±)

It will be seen that some multiple of a quantity lying between 13 and 14 would fit every case with the exception of one, in which the figure may have been slightly less than 13. Furthermore, let us add Miss King's two cases for the rat. In one of these, the second-born brood was delivered "about 14 days" (but not more than 14 days) after the first. In the other case, the second delivery was, she says, "about 12 days" after the first. It must be mentioned, however, that this "newborn" brood was actually found 13 days after the other.

Here, then, we have altogether 3 instances in which the broods were born about 13 days apart, 5 instances in which the interval was about twice as great, and 2 in which the interval was about 3 times as great. Statistically speaking, the case is not, of course, entirely convincing, and later findings may fail to conform to the scheme here indicated. But the probability seems to be sufficiently high to warrant its provisional acceptance.

Referring to the second table, it will be found that the intervals therein recorded could not all be interpreted as multiples of any one number, though four of them are not far from being multiples of 13. Were one disposed to have recourse to a not uncommon type of argument, he could point out that the normal variability of the single intervals might reasonably be expected to lead to a cumulative divergence in the course of five or six periods. But most of us would be reluctant to draw any conclusions from this second set of figures.

Regarding the existence of a definite periodicity in the ovulation of mice, there appears to be some difference of opinion. Most recent observers seem to agree that ovulation occurs independently of coition in the mouse and rat. Sobotta (1895), Kirkham (1910, 1913), and Long and Mark (1911) hold that it takes place not long after parturition. According to the latter authors the interval between parturition and the ensuing ovulation ranges from 14½ to 28½ hours. Their observations have thrown little light, however, upon the periodicity of ovulation at other times, though they do not seem to believe that this is governed by any great regularity. Sobotta (1895) states that ovulation recurs 21 days after parturition, basing his conclusions upon the examination of several females. He regards as improbable the occurrence of intermediate periods, at intervals smaller than this.

On the other hand, it is stated by Heape (1900) that "the usual length of the diœstrous cycle for rodents is ten to twenty days," that of the rat and mouse (in England) being given as approximately ten days (p. 26). Since it is also stated (Marshall, p. 135) that "in the mouse, the rat, and the guinea-pig, ovulation occurs spontaneously during 'heat,' and generally, if not invariably, during œstrus," it would follow that in these animals, ovulation likewise occurs at intervals of about ten days. This figure approximates much more nearly than Sobotta's to the thirteen-day periods indicated by my observations. Presumably, pregnancy would ordinarily interrupt these periods of ovulation, and initiate a new cycle at its close, though the phenomena of superfetation and deferred fertilization are pretty good evidence that this is not necessarily the case.

If, as seems probable from my data, the second brood of offspring resulted from a later period of ovulation, during or after the first pregnancy, we must suppose that the spermatozoa were retained in an active condition for many days and in some cases even for weeks. Fertilization was deferred until the arrival of viable eggs in the fallopian tubes or the uterus. If we consider the intervals (Table I.) between the last possible opportunity for copulation and the birth of the second litter, we have:[1] no. 1: 13 days ±; no. 2: 26 days; no. 3: 27 days; no. 4: 27 days; no. 5: 47 days (±); no. 6: 28 days; no. 7: 37 days; no. 8: 36 days.

Thus in only a single case (the first) could copulation have occurred as late as 22 days (*i. e.*, the normal period of gestation) before the birth of the second brood. In case no. 5, if we suppose that the fetuses developed at the normal rate, at least one spermatozoön must have retained its fertilizing power for 25 days or more. In cases 7 and 8, even if we admit the probability here of coition *after* parturition, the spermatozoa must have remained alive for 15 and 14 days respectively.

---

[1] In all these cases except no. 5 and perhaps no. 1, the first brood was born unexpectedly, and it is likely that the male was still in the cage at the time of delivery.

An alternative hypothesis would be to assume that *gestation* rather than fertilization had been retarded in these cases. Thus Schultze (1866), while not denying the theoretical possibility of superfetation, explains all of the reported cases in man on the basis of either the death or the retardation of the less advanced fetus. This would also seem to be the opinion of Godlewski (1914), who, after pointing out what he believes to be the physiological impossibility of this process, makes the following dogmatic assertion: "Wir müssen demnach annehmen, dass die Eier, welche den Mehrgeburten den Ursprung geben, *gleichzeitig* befruchtet werden" (781). On this assumption, we should have to suppose that, at least in cases 1 and 5, the ova which gave rise to the second brood had been liberated and fertilized simultaneously with those which gave rise to the first. In the remaining cases, on the other hand, they may have been liberated and fertilized after the birth of the first brood.

Now Daniel (1910) has shown for white mice and Miss King (1913) for white rats that parturition may be considerably deferred when the mother is still suckling an earlier brood. This retardation was found, in some cases, to be as much as 10 days for the mouse and 12 days for the rat. Both of these writers assume that fertilization ensues fairly promptly after successful insemination, and that the delay in parturition is due entirely to a prolongation of embryonic development.

If we attempt to apply this interpretation to some of the present examples, we are led into difficulties even greater than those which confront the hypothesis of deferred fertilization. For example, in case 5 (Table I.), the last opportunity for copulation was 19 days before the birth of the first litter, and about 47 days before the birth of the second. Even on the assumption that the fertilization of the second lot of eggs actually took place as late as this last day, the period of gestation was prolonged by about 25 days, *i. e.*, the normal term must have been more than doubled. Now the first brood consisted of only two individuals, so that any retarding effect due to lactation would have been almost at a minimum. Daniel found that this amounted to only about a day for each young mouse suckled.[1]

If it be contended that the retardation probably resulted from the *gestation* of the first litter rather than their subsequent nursing, it is relevant to point out that this species of *Peromyscus* may give birth to 5, 6, or even 7 young, with little, if any, prolongation of the period of gestation beyond the normal.[2] Again, in cases 7 and 8, the birth of the second brood occurred 37 and 39 days respectively after the first. In both of these cases it is possible that copulation occurred during or shortly after the first pregnancy, since the male was not removed until after the delivery of the first brood. Thus, at the least, we must assume that the second gestation was prolonged more than two weeks, owing to the suckling by the mother of her three earlier young.

To me it seems much more credible that the cases here discussed resulted from the fertilization of recently liberated eggs by spermatozoa which had been received many days previously. I do not think it helps us at all to assume that the term of development, after fertilization, was prolonged to any such extent as we should here have to suppose. Nor do I believe that it is necessary to assume a *second* copulation to account for the later brood, though this possibility is not excluded in my experiments. To refer again to case 5, of my first table, the last opportunity for copulation was 19 days before the birth of the first brood, *i. e.*, only 3 days after the conception which initiated the development of this brood. Is it not as easy to admit the retention of living spermatozoa from this first effective copulation as it is to admit their retention from another copulation occurring only three days later?

At first glance, it is not clear why these cases of deferred fertilization should, in every instance, have followed a previous pregnancy. In other words, why should not this phenomenon have appeared in females which had earlier merely undergone insemination, without a normal conception having first ensued? As a matter of fact, I have never found an instance, among all the births recorded, in which a first brood[1] is known to have been born more than 22 days after the last possible opportunity for coition. Why should not the spermatozoa have occasionally been held in reserve for a period of ovulation occurring some time after this last opportunity for copulation? In reply, I can only point out that coition probably occurs only when the female is in a condition of "heat." If, on the advent of the first "heat" period after the introduction of the male, fertilization did not follow insemination, it would, in a large proportion of cases, point to a sterility (temporary, at least) on the part of one or both sexes. As a matter of fact, my records show that the great majority of the females which conceived at all did so during the first half of the sojourn of the male in the cage.[2] As stated above, only one female in the course of my experiments is known to have become pregnant as late as the last possible date of copulation.

---

[1] For rats, indeed, Miss King found that retardation was not certain to follow unless more than five young were suckled.

[2] One brood of 6 was born 23 days after the earliest possible fertilization, while one brood of 6 and one of 7 were born 24 days after the earliest possible fertilization.

To what degree the term *superfetation* is applicable to the cases comprised in my first table is a matter of definition. Case no. 1 (as well as the two cases described by Miss King) would doubtless be covered by the term as ordinarily understood, since one period of gestation, with little doubt, was superimposed upon another. For reasons stated, it does not seem likely, however, that two sets of developing fetuses were simultaneously present in any of the other cases. But in at least one of these instances (no. 5), the sperm which fertilized the second lot of eggs must have been received during or prior to the first pregnancy. Perhaps the term superfetation might conveniently be extended so as to cover such cases as these last. But the word is plainly inapplicable to instances in which the second effective copulation occurred *subsequently* to the delivery of the first brood.

Admitting the last named possibility for most of the cases comprised in my Table I, we none the less find in them instances of *deferred fertilization*, a phenomenon which has been little recognized among mammals. It is stated by Marshall (1910, p. 136), on the authority of various investigators, that "in certain bats copulation is performed during the autumn, whereas ovulation is postponed until the following spring, the animals in the meantime hibernating, while the spermatozoa are stored up in the uterus." There is no inherent improbability of the occurrence of parallel phenomena in rodents. The presence in the uterus of active spermatozoa long after copulation ought, however, to be directly determined for these animals.

---

[1] *First*, as distinguished from the supernumerary broods here discussed. I do not refer necessarily to the first offspring of a given mother.

[2] This commonly covered 20 days.

A few additional comments seem worth while before closing these notes. It must be pointed out that, in *Peromyscus*, at least, the phenomena discussed cannot be of very rare occurrence. The seven cases in the first table which relate to deer-mice have been observed in the course of rearing about 250 broods of young. Thus nearly 3 per cent. of the litters born were followed by these deferred or supernumerary broods. Whether or not the small size of these first litters (1 to 3 young) is a matter of significance I cannot conjecture.

Furthermore, it should be stated that most of these supernumerary litters comprised normal, healthy animals, more than 80 per cent. of which survived nearly or quite to maturity. In the great majority, at least, of the cases recorded for man, one, if not both, of the fetuses has been dead or imperfectly developed when born.

Again, it is worthy of remark that in four cases out of eight the parents of these supernumerary broods were both very young mice, which had not yet been separated according to sex, while in two more cases the fathers (though not the mothers) were very young animals. Since the proportion of all my broods born of parents of such an early age is very small, it seems likely that this fact is of some significance.

Finally, the possibility suggests itself that some of the alleged instances of "telegony" which are recorded from time to time, may result from the actual retention of spermatozoa received from an earlier mate. A later copulation with a different partner might happen to coincide with a conception in which the earlier insemination was really the effective one.

LIST OF WORKS CITED.

Aristotle.

a De Generatione Animalium (Translated by Arthur Platt). Oxford, Clarendon Press, 1910. (IV., 4: 773 b to 774 a.)

b Historia Animalium (Translated by D'Arcy W. Thompson). Oxford, Clarendon Press, 1910. (VII., 4: 585a.)

Daniel, J. F.

'10 Observations on the period of gestation in white mice. Journal of Experimental Zoölogy, vol. 9, no. 4, Dec. 1910, pp. 865–870.

Godlewski, E.

'14 Physiologie der Zeugung. (In Bd. III. of Winterstein's Handbuch der vergleichendenden Physiologie). Jena, 1910–1914, pp. 457–1022.

Heape, W.

'00 The "sexual season" of mammals and the relation of the "pro-oestrum" to menstruation. Quarterly Journal of Microscopical Science, vol. 44 (N. S.), no. 173, Nov., 1900, pp. 1–70.

King, Helen D.

'13 Some anomalies in the gestation of the albino rat (Mus norwegicus albinus). BIOLOGICAL BULLETIN, vol. XXIV., no. 6, May, 1913, pp. 377–391.

Kirkham, W. B.

'07 The maturation of the mouse egg. BIOLOGICAL BULLETIN, vol. XII., no. 4, Mar., 1907, pp. 259–265.

Kirkham, W. B., and Burr, H. S.

'13 The breeding habits, maturation of eggs and ovulation of the albino rat, American Journal of Anatomy, vol. 15, no. 3, Nov., 1913, pp. 291–317. pls. 1–6.

Long, J. A., and Mark, E. L.

'11 The maturation of the egg of the mouse. Carnegie Institution Publication no. 142, 1911, 72 pp., 6 pls.

Marshall, F. H. A.

'10 The physiology of reproduction. Longmans, Green and Co., 706 pp.

Schultze, B. S.

'66 Ueber Superfoecundation und Superfoetation. Jenaische Zeitschrift für Medicin und Naturwissenschaft, Bd. 2, 1866, pp. 1–22, pl. I.

Sobotta, J.

'95 Die Befruchtung und Furchung des Eies der Maus. Archiv für mikroscopische Anatomie, Bd. 45, pp. 15–93, pls. II–VI.

FURTHER DEVELOPMENTS IN OVARIOTOMIZED FOWL.

H. D. GOODALE,

MASSACHUSETTS AGRICULTURAL EXPERIMENT STATION.

In several castrated Brown Leghorn females certain developments of particular interest have taken place. These developments relate not only to the plumage and other external characters but also to certain structures associated with the reproductive organs. In brief, these individuals developed male plumage and other male characters. After a time, however, certain changes in the plumage of some individuals took place, best described as a change to or toward the female type, as the case might be. Still later, the plumage changed again to or towards the male type. Usually, the development of female plumage in poullards is to be referred to a regeneration of the ovary but an examination showed that no regeneration had occurred in these individuals but that instead an organ *sui generis* had grown. A portion of the organ has been removed from each bird and sectioned. Its structure clearly is neither that of the ovary nor that of the testes. The exact nature of these organs cannot be determined at present, although their structure suggests that they have some relation to the epididymis. On the right side in normal females, both ducks and fowl, a bit of tissue is sometimes found on the spot corresponding to the site of the ovary on the other side. From this body a strand can sometimes be traced posteriorly. While this body has some resemblances histologically to that of the organs developed in the castrated females, it is impossible to assert that the latter results from the hypertrophy of the former, even though there are many other reasons for drawing this conclusion. To demonstrate the assumed relationship between the structures will require a considerable series of stages which are not at present available and whose collection will require some time.

The history of these cases may now be considered in detail.

The first is that of the pullet described in the *American Naturalist*, Volume XLVII., 1913. The chick had been completely castrated when three weeks of age. In due course of time, the bird developed a good male's plumage with large comb and spurs. However, there were a number of feathers in the dorsal region which were shaped and stippled like those of the hen but rather different in color. (Fig. 3, *b*, *American Naturalist.*) With the coming of the moult it was found that the new feathers were essentially like the old. That is, the plumage retained its intermediate character. The bird was then killed and dissected. The conditions found were so remarkable that it was thought best to await confirmatory data before publishing. On either side, at the level of the adrenals was an organ which had the appearance of a small testis, though divided into several lobes. Histologically, however, it was very different. Leading posteriorly from each of these structures to the cloaca was a yellowish white strand (cord, duct) resembling an immature vas deferens. The *left oviduct* in an infantile condition was present.

The presence of the bodies described for 1196 appears to be more usual in ovariotomized fowl because such organs, with one possible exception, have not been found among the eight or ten ovariotomized ducks that have been opened or autopsied at various times, though found in all fowl thus far examined. Both species have ranged from a few months to three years of age, all but one at least a year old. Evidently the possibility of the development of the bodies in question rests upon some genetic basis. Nor are they necessary for the assumption of male characters by the ovariotomized female, since the ducks have developed as good male plumage as the fowl.

Perhaps the question will be raised regarding the possibility that all these individuals were really males. It is desirable to consider this phase of the matter in some detail. There are two general possibilities of error—first, a possibility that an error was made in identifying the sex of the individual at the time of the operation; an error however, that would be equivalent to one made in identifying the sexes of domestic poultry by their plumage. The gonads of the male and female are quite unlike, even before hatching time (cf. Thompson, *Arch. Ent.*, 1911). The *single* ovary is a flat sheet of tissue, roughly triangular in outline, found on the left side only. The *two* testes are each cigar-shaped.

The second possibility is that a clerical error was made. It should be needless to say that great care is taken to avoid this sort of mistake. However, there are several checks on mistakes of either sort. First, in ovariotomy an incision is made on the left side only, never on the right. In the extremely rare event of there being an ovary on the right side its presence would remain unknown until much later. The right side is never examined at the time of operation. Therefore, if a supposed female were really a male, the presence of the right testis would bring the mistake to light in due course of time. Second, an infantile oviduct has always been found in the cases autopsied. These oviducts are not a vague strand of tissue but on the contrary are in about the same condition as a four or five month pullet's before the ovary has begun to enlarge preparatory to laying. Third, there are a number of peculiarities about the castrated females that differentiate them from males both normal and castrated. These are fully considered in another place.[1] Finally, it may be noted that the number of instances on record is fairly large. During the past year a flock of fifteen ovariotomized ducks, besides several ovariotomized fowl, have been maintained at this station.

The second individual for consideration is a rose-combed bird of a different strain of Brown Leghorns which was castrated as a four-weeks-old chick in 1914. This bird, number 3840, developed a beautiful male plumage in due course of time, except that for some reason the development of the tail was imperfect. The main tail feathers were present but had an injured appearance. Number 1196, described above, likewise had the dorsal portion of the uropygium missing, which gave her a bob-tailed appearance. Number 3840 also had the same bob-tailed appearance in 1914, though the feathers were actually present, but lost it with the new feathers in the autumn of 1915. The comb and wattles developed more slowly than those of the normal male, but by late spring they had become large and male-like. The spurs also were fully developed. When the bird moulted (1915) the new feathers were those of the *female* throughout. As is frequently the case, the bird moulted piece-meal, both kinds of feathers co-existing at one time. Gradually, however, the male feathers were replaced by feathers that could not be distinguished from those of the female. When it is recalled that the Brown Leghorns are practically identical in color with the Jungle fowl, the change can readily be appreciated. The bird was not killed as it was desired to keep her for further observation, but instead she was opened on each side. Organs roughly similar to those described for 1196 were noted. On the right side a strand of tissue was traced posteriorly for a few millimeters but no strand could be identified on the left. No trace of anything resembling normal ovarian tissue was found. As the location of the incision was unfavorable for finding the oviduct, this was not attempted. A bit of each of the organs were removed and sectioned. The histological findings were like those described below.

---

[1] Paper in press.

A short time after the operation it was noted that the new saddle feathers that came in were male-like in that they were fairly long, and laced with bright yellow, though rather bluntly pointed at the end and the central stipe though nearly black was often sprinkled with brown to a greater degree than usual for a male. The feathers of the tail coverts particularly contained much brown. In the breast feathers, less change was noticeable, most of the feathers remaining deep reddish salmon though some of the feathers contained some black. Other parts of the bird showed feathers much like those of the female type.

The third instance is that of a bird hatched June 22, 1913, and castrated August 8, 1913. The juvenile female plumage was well developed at the time. By early winter, however, this plumage had given place to one nearly or quite identical with that of the young male. The spurs developed fully but the comb never became really male-like, but had rather the general appearance of a Leghorn female's comb when straightened up. This comb, however, never loped but was always erect, an exception not at all rare among Leghorn females. It was anticipated that when the bird moulted during the summer the full male plumage would be assumed. Instead, a different type appeared. The feathers were shaped like those of the hen, except the tail coverts which in shape resembled those of an English Campine male. That is, they were rather longer than those of the hen, curved, and with rounded ends. The dorsal feathers were dull black with golden shafts, sometimes with a few minute brown spots on the margins. Ventrally the feathers were black.

With the moult of 1915, a further change took place in that the breast feathers were replaced with salmon-colored feathers, while there was some increase in amount of brown stippling dorsally, so that this bird, too, was very much like a female. She was opened on each side in October, 1915. The same set of organs was found as described for the preceding instances. This bird, like 3840, also changed the character of its plumage after the operation. The new breast feathers were black, while the saddle feathers were good male though not as long and pointed as is usually the case.

A fourth instance has a history somewhat different from the one just preceding, although it was a litter sister and castrated at the same time. The castration, however, proved to be incomplete in that regeneration of the ovary took place. A minute bit of the ovary must have been left and when it became sufficiently large the new feathers that developed under its influence were female. Nearly a year after the first operation, a second was made and an attempt made to remove the regenerated ovary. Apparently it was successful for the bird soon after began again to assume male characters. The spurs became long but the comb has always remained hen-like, though erect. The present plumage is a mixture of male and female characters. The breast feathers are almost black but contain a little salmon in small patches. The dorsal feathers are not much longer, if any, than those of the normal female but they are triangular at the apex and have a margin of golden bristles. The centers, however, are *female* colored. An examination of the right side in October, 1915, showed a body similar to those described but rather larger. The left side seen from the right appeared to be empty but when an attempt was made to open the bird on the left side, unexpected difficulties were met, so that it was deemed desirable to proceed no further.

There remain two other birds to be considered. Though both were of hybrid origin their plumage was that of a typical Brown Leghorn. At the time they were castrated, the female juvenile plumage was well developed. After castration both developed spurs and a perfect male coat of plumage but their combs remained small.

Number 4290 was killed November 25, 1914. The same sort of organs on the site of the gonads were noted again. The other, 4471, is still alive and in *perfect male plumage.* In the summer of 1913, a female-like plumage developed, followed in the fall by a return to the male plumage. In 1915 no change in plumage color took place. When opened, October 11, 1915, the same sort of organs were found. Thus, in all but possibly one instance there has been a development of glandular material on *both* sides. The exception relates to the individual that was examined on only one side. It seems probable that though numbers 3840 and 2087 were operated on just prior to their return to the male condition the operation as such had nothing to do with the results secured but rather that a change to this plumage would have taken place as in the case of 4471. It is of course possible that the operations accelerated a change about to take place. It is quite possible, too, that the changes in plumage are cyclic in nature, like the occurrence of the summer plumage in the normal drake.

Because it is desired to keep the birds alive for further observations, the structure of the organs cannot at present be given in detail. It was thought at first that a small piece would suffice for a determination of its structure, but the material thus far examined indicates that the study will have to be made from the standpoint of the organ as a whole. Owing to lack of material, this cannot be attempted for some time to come. However, the material on hand is sufficient to indicate something of its nature. The following description is provisional: The histological findings vary from specimen to specimen and also in different parts of tissue from the same bird. The differences, however, are probably to be referred to developmental stages, though this is by no means certain. In what is supposed to be the early stages, the cells are small with relatively little protoplasm and closely packed. There is a very weak development of connective tissue which separates the cells into poorly defined groups. Intermediate stages can be found in which the groups of cells become well defined. After the first stage, there is a considerable increase in the connective tissue which in some instances appears to produce smooth, refractive rods or strands. Stages have been noted in which the central cells separate somewhat after the manner of thyroid tissue and stain less readily. These appear to be degenerating. Later the marginal cells also break down so that small open spaces may be found lined mostly by connective tissue. They in turn appear to coalesce by the breaking down of neighboring walls, so that large open spaces appear in the tissue, perhaps homologous with the vesicles filled with a straw-colored fluid observed macroscopically in some instances. In one part of the organ a group of tubules lined with a single layer of square cells has been noted. These appear to be of a different character from the spaces described above. They may contain a finely granular but otherwise amorphic substance.

It is rather probable that the present organ is the result of a development of the Wolfian body. This view, however, is merely a working hypothesis.

Should further investigation confirm this view, it may throw light on the structures described by various observers in cock-feathered females. These bodies are very likely identical with the bodies found in castrated females. Certainly the presence of true seminal tubules or spermatozoa together with ova must be demonstrated before similar individuals found in nature containing these organs can be designated as hermaphrodites.

An explanation of the plumage changes is mainly a matter of surmise. At first, one is inclined to lay the changes at the door of the new organ but since they do not appear in all individuals which have developed the organs, it is evident that if the organs are concerned with the changes there must be some change in the activities of the organs either preceding the changes in plumage or accompanying them.

Among ducks it has been noted that a similar change in plumage is associated with the testes in the *male,* although the organs described have not been found in several castrated female ducks which have been examined. The *ovariotomized* duck may or may not undergo a change in plumage, corresponding to that of the male. Those that undergo such a change have returned in due course of time to the breeding plumage. Thus their temporary plumage is like that of the summer plumage of the male.

The change of plumage in the ovariotomized fowl may be due to the release of a mechanism for changing the plumage but which has been hidden or rendered latent in some way during the phylogeny of the race or as a result of domestication. In this connection it may be noted that laying hens give evidence of a summer moult. This moult is rarely complete and is evidenced usually by the shedding of a comparatively small number of feathers.

THE BEHAVIOR OF THE ACCESSORY CHROMOSOMES AND OF THE CHROMATOID BODY IN THE SPERMATOGENESIS OF THE RABBIT.[1]

L. J. BACHHUBER,

University of Wisconsin, Department of Experimental Breeding.

The following studies resulted from a series of experiments to determine the effect of lead poisoning upon the germ-cells of the male rabbit as indicated by his offspring. My intention originally was to attempt to determine the manner in which the lead-poisoning affected the normal mitosis. The problem of the normal mitosis in itself proved to be so large that the study of the effect of lead-poisoning had to be postponed to a future time.

The following work was done under the direction of Dr. M. F. Guyer, to whom the writer is very much indebted for many valuable criticisms and kindly help. The writer is also indebted to the kindness of Professor L. J. Cole for aid given in getting the necessary material for this study.

All of the rabbit testes used were from animals raised at the barns of the Department of Experimental Breeding of the University of Wisconsin. These males were chosen from the normal stock resulting from the double matings described by Cole and Bachhuber (1914).

A fairly successful fixing reagent was found in Flemming's strong. This method brought out the chromosomal and cytoplasmic structures better than Gilson's, Zenker's, Hermann's and possibly Bouin's fixative. Bouin's gave good results in the study of the accessory chromosomes and the chromatoid body. A new method reported to be in use in McClung's laboratory was also tried with considerable success. This fixative employs urea as a means of more rapid penetration. To one hundred cubic centimeters of Bouin's, made up of seventy-five parts of aqueous picric acid with fifteen parts formalin and ten parts glacial acetic acid, were added one and one-half grams chromic acid and three grams of urea. The solution was then heated to thirty-seven degrees and the tissue added. This fixative made the chromosomes stand out somewhat better and clearer than any of the other fluids.

---

[1] Papers from the Department of Experimental Breeding, Wisconsin Agricultural Experiment Station, No. 6. Published with the approval of the Director of the Station.

The sections were cut from four to fifteen micra in thickness and stained in (1) Delafield's hæmatoxylin and eosin, (2) Haidenhain's iron-hæmatoxylin and acid fuchsin or orange G, (3) safranin with gentian violet or lichtgrün. Method (2) proved to be the most successful and gave some very excellent results. This method was most valuable in bringing out the detailed structures, especially the chromatoid body. Cytoplasmic structures were as a rule satisfactorily stained in Flemming's triple stain.

Smear preparations were also made. These were fixed in Flemming's strong or in Bouin's fluid. Haidenhain's iron-hæmatoxylin gave the most satisfactory result when counter-stained with lichtgrün.

Mammalian spermatogenesis seems to offer greater difficulties for study than any other form. This is due to the impossibility of securing by means of existing reagents and methods proper fixation. In nearly all preparations it has been found that the chromatic structures have a tendency to mass so that individual details are lost. The chromosomes in the rabbit have this to a very marked degree. The various stages in spermatogenesis were numerous enough, but a great many stages had to be examined in order to find those in which chromosome counts were possible.

N. M. Stevens (1911) in the spermatogenesis of the guinea-pig found that material "very unfavorable and the results are not so satisfactory as are desired." Montgomery (1912) in his work with the human spermatogenesis has to say practically the same thing, "the fixation was not as excellent as might be desired." Wodsedalek (1913), however, seems to have encountered less of the massing of chromosomes in the spermatogenesis of the pig than is present in other mammalian forms.

In both the rat and the guinea-pig, and later in the bull, the writer has found the same condition, although to a lesser degree than in the rabbit, namely, the strong tendency of the parts within the nucleus to mass, hiding many of the individual structures, and leaving much to be desired in the form of improved methods. In the rabbit, the chromosomes always agglutinated, permitting but few chromosomal counts. Because of this, the only stages in which chromosomal counts were possible with any degree of accuracy were the primary spermatocytes. In later stages it was decidedly more difficult to find stages in which counts were possible.

In the rabbit, the structures to be followed more readily are, first, the two accesory chromosomes and second, the chromatoid body. After the spermatogonial stages, the accessory chromosomes could nearly always be identified and rather easily traced. The chromatoid body, similar to that described by Wilson (1913), whose origin could not be determined, can be easily followed, beginning with the primary spermatocytes, and can even be identified when it is cast off from the transforming spermatosomes.

The other structures displayed in the spermatogenesis of the rabbit, and the various processes connected with them, do not differ materially from the corresponding stages described for other forms. In the following pages, the process of spermatogenesis, in so far as it could be determined, will be taken up in the order of the successive stages of spermatogonia, primary and secondary spermatocytes, spermatosomes, and the fully developed spermatozoa.

1. Zones of Proliferation.

Throughout all the tubules of the testes, there appear small areas in which active proliferation reveals almost every stage of spermatogenesis. These areas are not, however, confined to any particular section or part of the tubule. All indications of the active zones go to show that a certain area may be active for a period, then halt in its activity while a nearby area becomes active in spermatogenesis. There are thus, scattered throughout each of the tubules, areas of active proliferation and areas of rest. In each of these areas of activity usually there may be seen the ordinary arrangement of the spermatogonial cells on the outer margin with the primary spermatocytes adjacent to them. Further towards the center lie the secondary spermatocytes while the spermatids, the transforming spermatosomes, and the fully developed spermatozoa lie in the central cavity.

2. Spermatogonial Stages.

It has been difficult to obtain satisfactory preparations of the spermatogonial cells showing all of the essential structures, chromatic and achromatic. Nutritive cells were also comparatively scarce although a few may have been identified (Fig. 1). They are very similar to the resting stage of the spermatogonia with which they may easily be confused and their identity is never certain. The nuclei of these nutritive cells usually appear oval or irregular in shape and contain masses of chromatin scattered throughout This close resemblance to the nucleus of the resting spermatogonia makes it not at all improbable that the nutritive cells, if they are such, may be derived from the spermatogonia. Montgomery (1912) has found this to be true in man. He was able to trace directly the formation of the Sertoli cells from the spermatogonia by the presence of a rod-like body in the cytoplasm, which was, according to him, an invariable indication of the Sertoli cells. Hegner (1914) takes the view that the Sertoli cells arise from the primordial germ-cells. This may also be true in the rabbit although there is little direct evidence to support either view. The similarity of the Sertoli cells and the spermatogonia and the relative number of these cells affords the only evidence.

During the earliest prophases of the spermatogonial stage (Figs. 2, 3) the nucleus is somewhat elongated and irregular as a rule, and contains two or more large, spherical karyosomes. These may be the two accessory elements which can be traced very accurately after the formation of the primary spermatocytes. Small linin threads, somewhat granular in appearance, radiate out towards the periphery of the nuclear wall (Figs. 4, 5). Slender fibrillæ extend throughout the cytoplasm (Fig. 2). Between these are small areas of a granular appearing substance. The centrosome and the other cytoplasmic structures cannot be identified although some of the more granular parts of the cytoplasm may include the centrosome and the chromatoid body. This is however doubtful because the staining reactions given by these structures in the primary and secondary spermatocytes ought also be given in the spermatogonia.

Only one spermatogonial stage has been found in which the chromatoid body appeared to be present (Fig. 6). While it has the characteristic appearance of this body, it is a rather doubtful case because it could not be identified in any other of the spermatogonial stages.

As development continues, dense masses of chromatin appear concentrating along the radial linin threads. These masses vary in number, but never exceed the diploid number of chromosomes, making it highly possible that these masses later transform into the univalent chromosomes. The entire nucleus stains heavily with the basic dyes, indicating a large increase of the chromatin content of the cell. In this manner, from twelve to twenty-two masses of chromatin are formed, all united by heavy linin threads. Gradually the linin threads disappear, and the chromatin masses assume a more regular appearance (Fig. 7). The nuclear wall disintegrates as the chromosomes arrange themselves in the metaphase stage (Fig. 8). The spindle fibers are very indistinct although in favorably stained sections they may be made to stand out more strongly. Because of this excess of stain, the cells which were stained heavy enough to make the spindle fibers appear more plainly were useless for the study of any of the other structures.

The chromosomes at best tend to mass together, and if not strongly destained, form a huge black mass in which nothing can be distinguished. In some of the metaphase stages it is possible to count twenty-two chromosomes (Figs. 8, 9, 10). The X and the Y elements which are shown to exist from their subsequent behavior, could not be distinguished from each other or from the remainder of the chromosomes in the spermatogonial division stage. It appears probable that the two large karyosomes present in the early spermatogonial stages may represent the accessory elements because they retain their individuality and later appear to transform directly into two chromosomes of the spermatogonial division. At times they may be traced because of a slightly more rounded form than the other chromosomes. The chromosomes in general are elongated in shape although the tendency is towards a spherical form.

During the anaphase (Fig. 11), the divided chromosomes move toward opposite poles. As soon as the chromosomes reach the pole, they go into a resting stage, the new nuclear wall appearing immediately. The new cell walls may not appear until late in synezesis. The outlines of the new nuclei are first somewhat elongated, conforming to the rough outline of the massed chromosomes. Gradually these assume a more spherical shape, the spindle-fibers disappear, and the primary spermatocytes are ready for the growth period.

3. Early Maturation Stages.

While the number of chromosomes is not large in the rabbit, the difficulties encountered in counting chromosomes were great indeed. In all of the material, the tendency of the chromosomes to mass was, as already mentioned, very much in evidence, and the most careful tecnhique in fixing and staining did little to make the results more satisfactory. There were enough cells in which chromosome counts were possible to place the probable number as twenty-two in the spermatogonia and twelve in the primary spermatocyte.

It was found that twenty-two chromosomes went to each pole in the spermatogonial division. After synapsis has taken place, the number is twelve, showing that two of the elements remain single. These two elements are the accessory chromosomes. Thus the twenty ordinary chromosomes of the spermatogonia reduce to ten bivalents in the primary spermatocytes, while the two accessories do not undergo synapsis. As will be seen, the later behavior of these univalent chromosomes is entirely distinct from that of the others.

The early prophases of the primary spermatocytes show all but two of the chromosomes which passed to the poles in the spermatogonial division to grow irregular in shape, and finally weave out into fine strands which immediately spread throughout the nucleus (Fig. 12). All of the irregular masses spin out into fine threads constituting the leptotene threads, leaving two, still rounded chromosomes, which can from this time on be identified as the accessories. The leptotene threads, in both sections and smears, are seen to persist as independent units. Their exact number could not be determined because of their extreme length.

During this stage, the accessory elements remain as small spherical masses which can easily be identified if the mass of leptotene threads is sufficiently destained. During the process of synapsis also, the X and the Y remain unchanged, always close together, and sometimes apparently connected by thin, dark-staining threads of chromatin material (Fig. 15). No instances have been found which at this stage show the accessory elements in different portions of the cell.

In these prophases, the presence of the chromatoid body becomes definitely established. It stains similarly to the chromatic material in the iron-hæmatoxylin, the Delafield's hæmatoxylin, and in the Flemming's triple stain. Just where this body arises is still a question. As before stated, it has been found in only one spermatogonial cell and that was a rather doubtful case. In the primary spermatocytes it is absolutely constant (Figs. 16, 20, 22). In a few rare cases, two and sometimes three have been found, but these extra bodies were always very small and disappeared in later stages. The large body can be traced through the remainder of the process of spermatogenesis. Further mention of its behavior will be made later.

In the synezesis stage (Figs. 13–16), the threads drift and mass at one pole of the cell into a grouping which has apparently no established order and from which nothing definite could be determined. Occasionally the threads seem to have a parallel arrangement suggesting parasynapsis, but when they come out of the synezesis stage, considerable evidence points to a chiasmatype synapsis (Figs. 18, 19). The threads are wound around each other, making possible a division later in which each of the haploid chromosomes would be made up of alternating segments of chromatin material, from the respective leptotene threads of the conjugating pair. That the threads still retain a constancy in number seems highly probable for in favorably stained sections where their ends can be seen, the number never exceeds double the number, minus four, of the chromosomes of the primary spermatocyte.

Previous to the pachytene stage the cells have increased but very little in size. Now they begin to grow rapidly, complete fusion occurring between the conjugating threads, and all gradually drift back to the center of the cell, there to spread slowly throughout the nucleus. The threads enlarge, become lighter in staining capacity, due perhaps to the greater volume occupied by the pachytene thread, and finally form the large spireme (Figs. 17, 20, 21).

This stage of the primary spermatocytes, with the possible exception of the synezesis stage, persists longer than any other stage of the spermatogenesis. This conclusion is reached because these stages of the primary spermatocytes can be found in practically any portion of the tubules. Numerous as they are, it is extremely difficult to gain any knowledge of the processes in progress in them.

The accessory elements still retain their spherical form, usually lying closely side by side, but at this time occasionally in different portions of the cell (Figs. 17, 21). The chromatoid body enlarges and seems to become more prominent. At the same time it seems to acquire an activity in the cell although its function could not be determined.

4. Reduction Division.

The spireme of this stage in general appeared to be continuous, although some of the smears made under considerable pressure gave indications of segmentation (Fig. 23). The large spireme now condenses and soon forms the individual chromosomes. Up to this period of condensation the chromomeres stand out very distinctly (Figs. 18, 19). These condense to form the large mass of bivalent chromosomes which, because of their agglutination, usually stain so dense that the individual structure is entirely lost.

The X and Y elements, however, can nearly always be identified in these stages. As soon as the bivalent chromosomes are formed, they migrate into the equatorial plate. The accessory elements are nearly always a little to one side of this plate

(Fig. 23), usually close together. It may be that this position is constant, but the massing of the chromosomes hides the accessories (Fig. 24). In the division, the X and Y chromosomes move towards the poles long before the division of the ordinary bivalents (Figs. 25–29). The X is thus drawn to one pole, the Y toward the other. In this manner, each of the secondary spermatocytes will contain only eleven chromosomes, ten ordinary and one accessory (Fig. 30). The ordinary chromosomes, in their bivalent condition, are approximately twice the size of the spermatogonial chromosomes.

The split in the bivalent chromosomes is rarely in evidence except immediately preceding the division, although in a few isolated cases a V-shape becomes noticeable.

When the chromosomes reach the pole the chromatoid body seems to acquire more activity. During the formation of the equatorial plate, this body migrates close to the densely crowded chromosomes (Figs. 24–27). As soon as these start moving toward the poles, the X and Y preceding, the chromatoid body moves in between the two sets of chromosomes, lying approximately midway between the two centrosomes (Fig. 31). Just before the new cell wall is formed, this body moves into the cytoplasm of one of the newly formed spermatocytes. The possibility presents itself that the chromatoid body always follows either the X or the Y elements. The two accessories are, however, so closely related in size that it has been impossible to determine whether there is any such specific association.

Occasionally, in the division of the primary spermatocytes, there is found evidence that the accessory elements undergo a precocious division. The accessories lie in the plane of division and under certain conditions, they divide, forming two X and two Y elements. The division then proceeds in the regular manner with this exception: two X and two Y chromosomes go toward the poles, again preceding the division of the ten ordinary chromosomes (Figs. 32, 33).

5. SECONDARY SPERMATOCYTES.

The division of the secondary spermatocytes proceeds without any appreciable rest stage. In the telophases of the preceding division the nucleus is somewhat crescent shaped, but soon assumes a spherical form. This stage develops no spireme or leptotene threads. The chromosomes, eleven in number, immediately migrate into the equatorial plate to form a very dense mass which at times appears almost homogeneous, so closely are the chromosomes drawn together (Fig. 34). In this division the chromosomes, in migrating toward the poles, often appear as solid black ring-like masses with apparently no segments representing the individual elements. Dense fibers seem to be interwoven in the mass of chromosomes (Figs. 35, 36). It is seldom that the chromosomes can be distinguished in these stages. The X and the Y elements also as a rule lose their identity, but occasionally they can be identified by their presence in the center of the ring, connected by chromatin strands to the ordinary chromosomes (Figs. 37, 38).

The chromatoid body appears to undergo no division in these stages, following either one or the other of the chromatin masses. We thus find that approximately one-fourth of the total spermatids contain the chromatoid body.

6. Transformation of the Spermatids.

As soon as the division is complete, a new nuclear wall is immediately formed. The chromosomes as such disintegrate but the chromatic material soon condenses at the periphery of the nucleus (Figs. 39, 40). In this manner nearly all of the chromatic material is condensed, leaving one part which in every case is found somewhere near the center of the cell (Figs. 41, 43) and which may represent the accessory element. Sometimes there are one or more smaller bodies which may be remnants of the disintegrating ordinary chromosomes because they do not persist for any length of time. The large central body can be traced through the stages of further condensation and almost to the completely developed sperm.

After all of the chromatic material has condensed, the centrosome makes its appearance and moves close to the nucleus (Fig. 41). It here gradually becomes immeshed in strands of chromatic material within a slight infolding of the periphery of the nucleus. By a continuation of this process it soon becomes entirely surrounded by nuclear material (Figs. 41, 42, 43). The entire nucleus then migrates to one side of the cell and gradually leaves behind all, or very nearly all, of the cytoplasm, together with the chromatoid body (Figs. 43, 44, 45). At the same time the entire nucleus begins to condense and finally forms a flat plate, which has at times, however, the appearance of a convex plate (Fig. 46). In the meantime the sperm tail has been formed from the cytoplasm with the centrosome in the neck. The nucleus, now the sperm head, gradually enlarges with a resultant diminution of staining capacity. In properly stained sections and smears there can occasionally be distinguished a number of darker staining bodies one of which is usually larger than the others. The number of these approaches very nearly the number of chromosomes which went into the sperm head. It is thus possible that the chromosomes retain their individuality even in the fully developed spermatozoa (Figs. 47, 48). As the sperm head enlarges, these dark-staining bodies gradually diffuse through the entire head (Figs. 49, 50). Further enlargement brings about the complete development of the spermatozoön. The head manifests an even staining capacity, the neck shows the presence of the centrosome, while the tail takes such a light stain that it is barely visible (Fig. 51).

7. Conclusions.

1. The number of chromosomes in the spermatogonium is probably twenty-two.

2. The number in the primary spermatocytes is placed at twelve.

3. The number in the secondary spermatocytes is placed at eleven.

4. Two accessory elements, an X and a Y, are present. One-half of the spermatozoa contain the X, and the other half, the Y element.

5. A chromatoid body is present. Its function was undetermined. It underwent no division, and was finally cast off with the excess cytoplasm in the metamorphosing spermatid.

BIBLIOGRAPHY.

Cole, L. J., and Bachhuber, L. J.

'14 The effect of lead on the germ cells of the male rabbit and fowl as indicated by their progeny. Proc. Soc. Exp. Biol. and Med., Vol. 12, No. 1, pp. 24–29.

Guyer, M. F.

'10 Accessory chromosomes in man. Biol. Bull., Vol. 19, No. 4, pp. 219–234, pl. 1.

Hegner, R. W.

'14 Studies on germ cells. Jour. Morph., Vol. 25, No. 3, pp. 375–499, pls. 1–10.

Montgomery, T. H.

'12 Human spermatogenesis. Jour. Acad. Nat. Sci. Philadelphia, Vol. 15, 2d ser., pp. 1–22, pls. 1–4.

Stevens, N. M.

'11 Heterochromosomes in the guinea pig. Biol. Bull., Vol. 21, No. 3, pp. 155–167.

Wilson, E. B.

'13 A chromatoid body simulating an accessory chromosome in Pentatoma. Biol. Bull., Vol. 24, No. 6, pp. 392–410, pls. 1–3.

Wodsedalek, J. E.

'13 Spermatogenesis of the pig with special reference to the accessory chromosomes. Biol. Bull., Vol. 25, No. 1, pp. 8–32, pls. 1–6.

EXPLANATION OF PLATES.

All drawings made with camera lucida. Spencer 2 mm. apochromat oil immersion objective and × 16 compensating eye-piece used. All drawings ar- shown at a magnification of approximately 1,800 diameters. Drawings are accurate with reference to the nuclear material and the chromatoid body and their position in the cell; the cytoplasm, however, is represented conventionally. *S*, Sertoli cell; *C*, chromatoid body.

PLATE I.

FIG. 1. Sertoli cell, showing the relation of the metamorphosing spermatosomes to the nurse cells.

FIGS. 2, 3. Early stages of the spermatogonia with the two large karyosomes. Fig. 2 also shows the slender fibrillæ in the cytoplasm.

FIGS. 4, 5. Later stages of the spermatogonial nuclei showing the linin threads radiating out towards the periphery of the nuclear wall.

FIG. 6. Shows the only spermatogonial cell in which a body was found which resembled the chromatoid body.

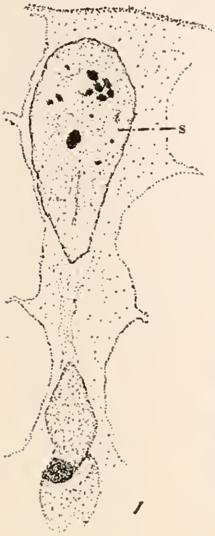
FIG. 7. In this stage a condensation of the nuclear material is taking place, forming masses which later transform directly into the chromosomes of the spermatogonia.

FIGS. 8–10. Metaphase stages of the spermatogonia showing the probable twenty-two chromosomes. Fig. 10 is taken from a smear preparation.

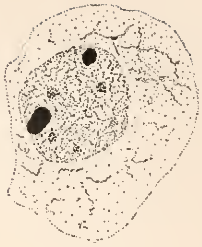
FIG. 11. Shows the divided chromosomes passing towards the poles. The X and the Y elements cannot be identified at this stage.

FIG. 12. Prophase of the primary spermatocyte showing the chromosomes from the previous division weaving out into fine strands which immediately spread through the nucleus.

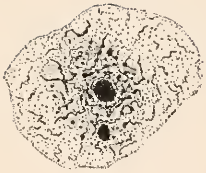
FIGS. 13–15. Synezesis stages of the primary spermatocytes showing the massing of the fibers, accompanied by a slow condensation, as shown in Fig. 15. This also shows the two accessory elements, which retain their spherical shape, while the ordinary chromosomes weave out into the leptotene threads.



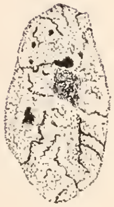
1



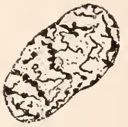
2



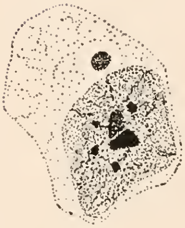
3



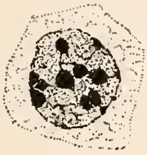
4



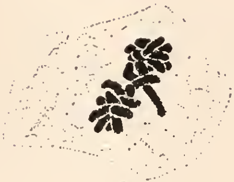
5



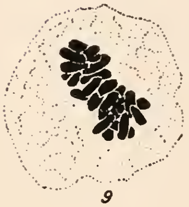
6



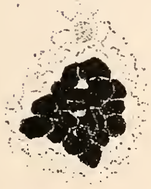
7



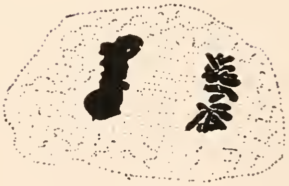
8



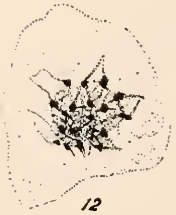
9



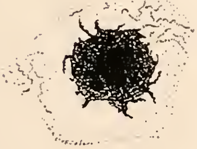
10



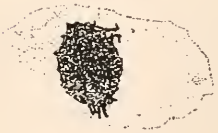
11



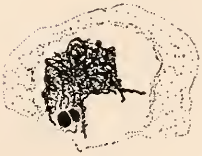
12



13



14



15

L. J. BACHHUBER.

PLATE II.

FIG. 16. Shows a further condensation of the leptotene threads, which finally results in the large spireme. This stage also shows the first appearance of the chromatoid body.

FIG. 17. The two accessory elements lie a little to one side of the condensing leptotene threads. The accessories can nearly always be identified in these stages.

FIGS. 18, 19. These stages show the leptotene threads coming out of the synezesis stage and giving evidence of a chiasmatype synapsis.

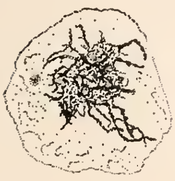
FIGS. 20, 21. The accessory elements are very conspicuous in the large spireme stages.

FIG. 22. Cell from a smear, showing evidence of a segmentation of the large spireme.

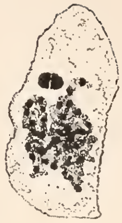
FIG. 23. The accessory elements lie a little to one side of the chromosomes massed in the equatorial plate stage, just previous to division. The chromatoid body has migrated into the same plane as the chromosomes.

FIG. 24. Shows the massing of the chromosomes, which usually hides the accessory elements. The chromatoid body is very prominent.

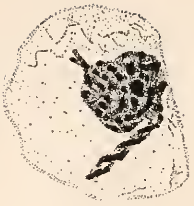
FIGS. 25–28. The two accessory chromosomes travel towards the poles long before the division of the ordinary chromosomes. The chromatoid body is still in the plane of the equatorial plate.



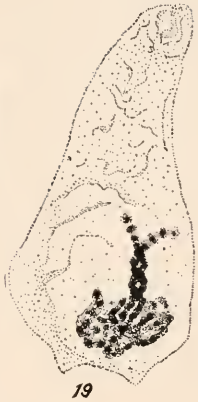
16



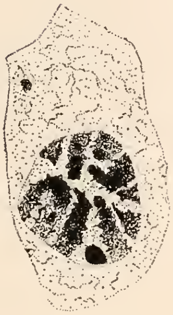
17



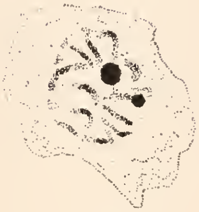
18



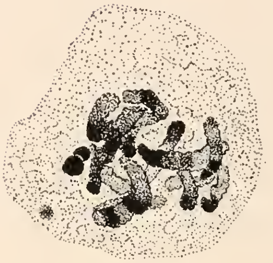
19



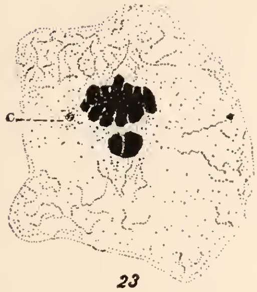
20



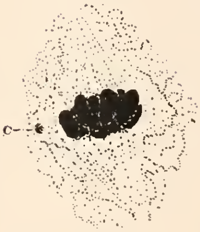
21



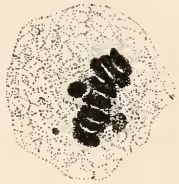
22



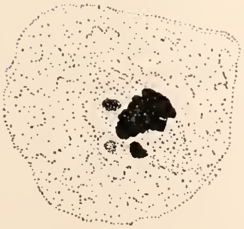
23



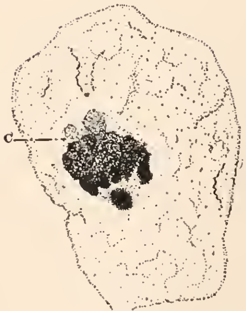
24



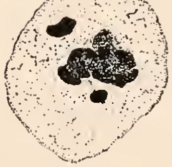
25



26



27



28

L. J. BACHHUBER.

PLATE III.

FIG. 29. Again showing the accessory elements migrating towards the poles in advance of the ordinary chromosomes.

FIG. 30. A stage following division of the chromosomes of the primary spermatocyte showing the eleven chromosomes at the pole, the accessory in the center.

FIG. 31. Shows the chromatoid body migrating in between the two sets of chromosomes immediately after division.

FIGS. 32, 33. These cells give evidence of a precocious division of the X and the Y elements. In this case, two X elements and two Y elements travel towards the poles.

FIG. 34. The chromosomes of the secondary spermatocytes have immediately lined up in the equatorial plate stage, ready for the next division.

FIG. 35. The chromosomes of the secondary spermatocytes divide and pass to the poles in ring-like masses, practically losing their identity as individual elements. This stage also shows the chromatoid body.

FIG. 36. Shows the chromosomes after reaching the poles and before formation of the spermatids. The chromatoid body is still present.

FIGS. 37, 38. Show the occasional identification of the accessory elements in the center of the closely interwoven mass of chromosomes, giving a ring-like appearance.

FIGS. 39, 40. Condensation of nuclear material at the periphery of the nuclear wall. Also shows the presence of the chromatoid body.

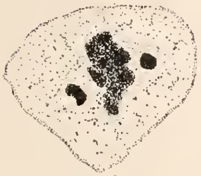
FIGS. 41–43. Further condensation of chromatin around the periphery of the nuclear wall. The centrosome is meshed in one side of the nucleus. The chromatoid body may lie in any portion of the cytoplasm and may be very irregular in shape.

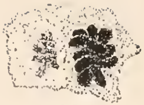
FIGS. 44–46. Still further condensation, with the gradual escape of the nucleus from the excess cytoplasm. The chromatoid body is cast off with the excess cytoplasm.

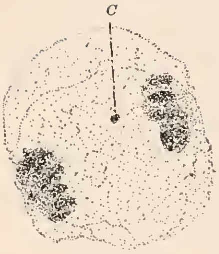
FIGS. 47, 48. Show the presence of darker staining masses of chromatin material which may represent the individual chromosomes in the sperm head.

FIGS. 49, 50. The darker staining masses now diffuse through the sperm head and finally form an even-staining mass of chromatin.

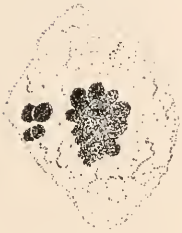
FIG. 51. Fully developed spermatozoön, showing the head, the middle-piece with the centrosome, and the long, thin, lightly staining sperm tail.

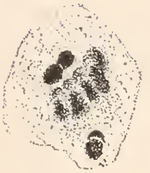

29

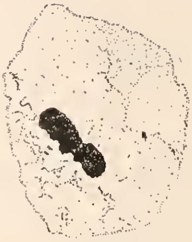

30

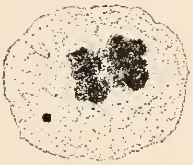


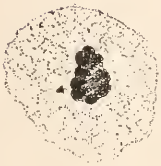
31

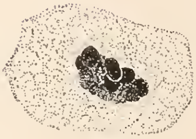

32

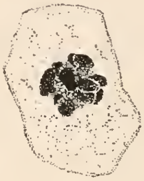

33

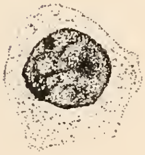

34

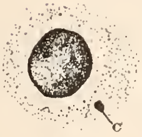

35


36

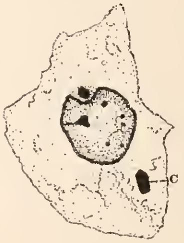

37


38

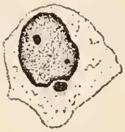

39

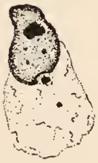


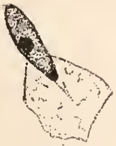
40

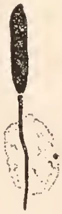


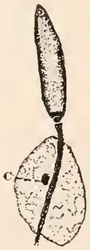
41


42

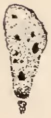

43

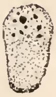

44

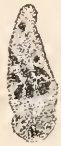

45

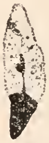


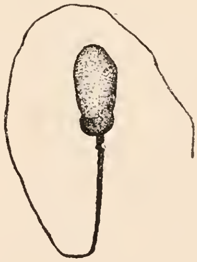
46


47


48


49


50


51

L. J. BACHHUBER.

Vol. XXX. *May, 1916.* *No. 5*

BIOLOGICAL BULLETIN

THE THEORY OF ANÆSTHESIA.

RALPH S. LILLIE,

Biological Department, Clark University, Worcester, Mass.

Anæsthesia, also termed narcosis, is a physiological condition in which the normal responsiveness or automatic activity of the living system—organism, tissue, or cell—is temporarily decreased or abolished. The subjective accompaniment of this change in higher animals is a more or less complete suppression of consciousness, with consequent insensibility to pain; the term "anæsthesia" refers more directly to this condition. By "narcosis" is usually meant a temporary paralysis or anæsthesia produced by chemical substances; this term has a more objective connotation; and is the one usually employed in purely physiological discussion. It is especially noteworthy that the condition may show all gradations of degree, ranging from a comparatively slight inhibition or insensibility to a state of profound depression in which the organism is completely inert and shows no response to even the strongest stimuli. Yet on the removal of the anæsthetizing agent the normal properties and activities return. *Reversibility* is thus an essential characteristic of the condition; this peculiarity distinguishes it from the irreversible change of death. There are, however, significant resemblances between these two states, and in fact transitions from the one to the other are frequent. Too prolonged or too profound anæsthesia may pass into death; and most anæsthetic substances, if present in too high concentration, soon cause irreversible and cytolytic changes in cells. There is in fact evidence that in many instances anæsthetic and toxic effects have the same essential physicochemical basis. The same cell-structures—especially surface-structures, *e. g.*, plasma-membranes—are primarily affected in both cases, but in the one case the change produced is reversible, in the other irreversible. The degree of reversibility is however itself subject to variation. In many colloidal systems changes which are reversible in their earlier stages may become irreversible later; and the fact that anæsthesia, especially if profound, cannot be prolonged indefinitely without danger to life, may find its explanation here.

In any theoretical discussion of anæsthesia it is important to recognize from the first that normal or physiological conditions of reversible inhibition or suspended activity are in no sense unusual among organisms. In both animals and plants irritability and automatic activity are fluctuating properties, with a wide range of strictly physiological variation. Thus in higher animals we have conditions ranging from the profound narcosis of sleep—a state due apparently to the accumulation of fatigue-products—to one of complete mental and physical alertness or wide-awakeness. Generally speaking, responsiveness is largely a matter of metabolic condition; and most vital activities are subject to inhibition or enhancement according to the physiological requirements. Variability of this kind is in fact a necessary condition of adaptation to the changing conditions of life. Thus the activities of animals as a class are influenced to a marked degree by variations in the food-requirements. In general they become sluggish and irresponsive when well fed, and show heightened activity when deprived of food. In other words, both the automatic motor activity and the responsiveness to the stimuli of food-substances—the physiological condition expressed in consciousness as *hunger*—are increased when the supply of energy-yielding material is depleted and *vice versa*. For example, the fresh water *Hydra* shows restless swaying movements when hungry; these movements increase the area swept by the tentacles, which respond promptly to the contact of small organisms or food-particles by capturing and conveying to the mouth.[1] When well fed the creature is quiescent, and the tentacles are indifferent to such contact; they are, as it were, in an anæsthetized condition; this state passes off as the organic demand for food reasserts itself. Such an instance illustrates the regulatory rôle which fluctuations in the general responsiveness of an animal play in its normal life. Similar variations of neuro-muscular responsiveness occur throughout the animal kingdom. This is well illustrated by sleep, which is an instance of a normal or "physiological" narcosis, characterized by a definite periodicity and by affecting especially certain parts of the central nervous system; the use of opiates illustrates how readily a chemically induced narcosis may pass into the physiological form. From such facts we must conclude that the essential basis of anæsthesia consists not in a purely artificial modification of nervous or other irritability, but in some normal or physiological modification which is capable of being intensified and prolonged by the use of certain physical and chemical agencies; these are the various anæsthetizing agencies, such as the electric current, cold, or narcotizing substances. From this point of view, anæsthesia is to be regarded not as an essentially abnormal or artificial phenomenon, but simply as an intensification of a normal physiological condition; and in investigating its essential conditions we are led first to consider the normal inhibitions and depressions shown by all living cells.

---

[1] *Cf.* S. J. Holmes, "The Beginnings of Intelligence," *Science*, N. S., 1911, Vol. 33, p. 473.

Instances of such normal inhibitions are innumerable. The motor neurones innervating any group of muscles become inexcitable during the activity of the antagonist groups, as Sherrington has shown; the respiratory nerve cells cease automatic activity with over-oxygenation of the blood; vasomotor, cardiac, glandular, and muscular activities are subject to various forms of inhibition, partly nervous and partly chemical in origin. Such inhibitory mechanisms play in normal life a part whose importance is daily more widely recognized by physiologists. Mechanisms of the inverse kind, which exercise sensitizing and reinforcing influence on various functions, are also frequent in organisms. A large part of these normal inhibitions and excitations are now known to be due to chemical substances (hormones) present in the blood and derived from ductless glands or other sources of internal secretion. The regulation and integration of bodily activities are thus largely under direct chemical as well as nervous control. Such normal chemical inhibitions are probably of the same nature as artificial inhibitions due to anæsthesia.

In both cases the same kind of physico-chemical modification in the irritable element appears to form the essential determining condition.

The phenomena of anæsthesia have thus the widest biological interest; they belong chiefly in the class of chemical inhibitions or desensitizations. The inverse phenomenon of sensitization—enhancement of irritability or responsiveness—is equally widespread and plays an equally important physiological rôle. Although its study has received less attention than that of anæsthesia, its physiological interest is no less great. Irritability may in fact be altered reversibly either in the direction of increase or decrease.

It is important to note that the same substance may cause either increase or decrease of irritability or spontaneous activity, according to the conditions of concentration, temperature, physiological state of the organism, etc. In the group of lipoid-solvent substances, which include most of the anæsthetics in common use, weak solutions very generally increase excitability; stronger solutions, within a certain range of concentrations, produce typical reversible narcosis; while still stronger solutions cause cytolysis. The basis common to all of these effects requires to be determined. The problem of the general nature of anæsthesia is in fact inseparable from the wider problem of the nature and conditions of irritability in general. The essential question may be expressed thus: what is the physico-chemical basis of this property of irritability, and what conditions determine its reversible increase or decrease by chemical or other agents? This problem is one of the most fundamental in biology; and the phenomena of artificial anæsthesia are of general physiological interest largely because of the light which they throw on this larger problem.

Instances of increase in irritability or spontaneous activity under the influence of low concentrations of anæsthetic substances are frequent in both animals and plants. One of the most familiar is the general nervous excitement caused by small doses of ether, alcohol and other narcotics. Automatic rhythmical activity, as of cilia, spermatozoa, or the heart beat, is very generally heightened in weak solutions of alcohol and other narcotics. The nerve-cells controlling the heart beat of *Limulus* show a faster rhythm in weak solutions of alcohol, chloral hydrate, choretone, and chloroform.[1] Hamburger has shown that many lipoid-soluble substances—iodoform, chloroform, turpentine, benzol, chloral hydrate, camphor, fatty acids, soaps—increase the amœboid and phagocytic activity of leucocytes, while stronger solutions decrease this activity.[2] A similar rule appears to hold for the respiratory center of vertebrates.[3] According to Vernon,[4] weak solutions of narcotics increase the consumption of oxygen in isolated tissues like the kidney. Tashiro and Adams find that low concentrations of urethane and chloral hydrate increase the excitability of nerve as well as its output of carbon-dioxide; in higher concentrations both are decreased.[5] The staircase phenomenon in irritable tissues is probably due to the stimulating action of small quantities of substances ("fatigue-substances") which in higher concentrations decrease irritability. Small quantities of alcohol increase the responsiveness of voluntary muscle and the energy of its contractions.[6] The musculature of medusæ shows increased response to mechanical stimuli in sea water containing a little alcohol.[7] Similar facts are met with in plants. Many depressant substances, when present in low concentration, increase the rate of growth.[8] Traces of ether have an accelerating or forcing influence on plant growth,—a fact of which practical use is made by horticulturists. Increase in oxygen-consumption under the influence of chloroform and ether has been observed by Elfving and others; higher concentrations decrease oxygen-consumption.[1] Demoor and others have observed an acceleration of protoplasmic rotation in plant cells during the early stages of chloroform and ether narcosis; alcohol also causes this effect.[2] Traces of ether increase the irritability of sensitive plants (*Mimosa*);[3] higher concentrations cause typical anæsthesia.[4]

---

[1] A. J. Carlson, *Amer. Journ. Physiol.*, 1906, Vol. 17, p. 182.

[2] Hamburger, "Archives Néerlandaises des Sciences Exactes et Naturelles," Serie III, B, 1911, p. 1; *Archiv für Anatomie und Physiologie*, Physiol. Abth., 1913, p. 77.

[3] *Cf.* Hamburger, "Koninklijke Akademie van Wetenschappen te Amsterdam," 1915, Vol. 17, p. 1325.

[4] *H. M. Vernon*, "The Function of Lipoids in Tissue Respiration," *Journ. of Physiol.*, 1912, Vol. 45, p. 197.

[5] Tashiro and Adams, *Internat. Zeitschr. f. physik-chem. Biol.*, 1914, Vol. 1, p. 450.

[6] *Cf.* Lee and Salant, "The Action of Alcohol on Muscle," *Amer. Journ. Physiol.*, 1902, Vol. 8, p. 61.

[7] *Cf.* Bethe, "Allgemeine Anat. u. Physiol. d. Nervensystems," Leipzig, 1903, p. 359. One half per cent. alcohol decidedly increases the mechanical irritability of the isolated central portion of the medusa *Cotalorrhiza*. F. S. Lee observed that in the Woods Hole medusa *Gonionemus* the spontaneous contractions of the swimming bell are markedly increased by small quantities of alcohol (1/16 to 1/4 per cent.); *cf. Amer. Journ. Physiol.*, 1903, Vol. 8, p. xix.

[8] Numerous instances of this effect are cited by Czapek, *Biochemie der Pflanzen*, Jena, 1913, p. 148.

A probably related phenomenon is seen in certain artificial modifications of response induced in various organisms by weak solutions of anæsthetics. A striking instance is the reaction of many lower animals to light. Loeb has found that *Daphniæ*, which normally show little or no directive light-response, become positively heliotropic in weak solutions of alcohol and other narcotics, in concentrations of a third to a half of those required for anæsthesia.[5] Similarly I have found that the larvæ of the marine annelid *Arenicola*, which normally exhibit strong positive heliotropism, become *negative* in weak solutions of various anæsthetic substances. Similar observations have been made by Torrey, A. R. Moore, and other observers.

The phenomenon of reversible *decrease* of activity or responsiveness is anæsthesia. The vital processes subject to such reversible arrest are of the most varied kind. They include amœboid movement;[1] protoplasmic rotation in plant cells;[2] all processes depending on response to stimulation, like muscular contraction and stimulation and conduction in nerve; automatic rhythmical activities like the heart beat or the motion of cilia or spermatozoa; cell-division;[3] the artificial initiation of development in unfertilized eggs;[4] the stimulating, cytolytic or other physiological action of salt solutions;[5] various fermentative and oxidative processes;[6] light-production, *e. g.*, by luminous bacteria;[7] typical metabolic processes like the assimilation of carbon dioxide by plants;[8] growth processes in plants and animals, and developmental processes dependent on growth and cell-division. It is especially worthy of note that not only motor activity and responsiveness are subject to control of this kind, but also processes like growth and development. The growth of seedlings may be temporarily arrested by ether in sufficient concentration, as Claude Bernard showed.[9] Cell-division in the eggs of sea-urchins is checked by anæsthetics in concentrations of the same order as those required for neuro-muscular anæsthesia in Arenicola larvæ.[10] It is thus not surprising that developmental processes, depending as they do on cell-division and growth, are similarly subject to inhibition by anæsthetics. Stockard and McClendon[1] have shown that such substances induce abnormalities like cyclopia in developing fish eggs, an effect which is to be referred to the arrested development of certain portions of the central nervous system, especially the anterior region of the fore-brain between the optic vesicles. Abnormalities of growth and development as well as of irritability may thus be produced under the influence of anæsthetics. Since an automatic power of growth—*i. e.*, increase in specifically organized and metabolically active material—is perhaps the most fundamental manifestation of vital activity, the fact that it is subject to reversible arrest by anæsthetic substances is of the greatest biological significance, and illustrates in a striking manner the unity of the conditions which control the most various cell-processes. We may infer that in the general course of constructive as well as of destructive metabolism, processes are concerned which are identical with those underlying the ordinary manifestations of stimulation. These latter, however, are almost certainly dependent on surface-changes, of which the most essential are probably variations in the electrical polarization of the plasma-membranes (see below, p. 365). The controlling influence of membrane-processes in such fundamental physiological activities as growth and assimilation is thus indicated by this susceptibility to arrest by anæsthetics.

---

[1] *Cf.* Czapek, *loc. cit.*, p. 159, for instances of this effect. Tashiro and Adams (*loc. cit.*) cite observations of Kosinski showing that respiration in yeast cells is increased in presence of 0.5 per cent. ether; 5 per cent. reduces respiration one half, while 7 per cent. almost stops it. Baer and Meyerstein find increased oxidation of oxy-butyric acid to acetone in the perfused liver under the influence of various compounds which in higher concentrations check oxidations, *e. g.*, tricholor-alcohol, *p*- and *m*-oxy-benzoic acid, *p*-oxy-benzaldehyde (*cf.* p. 458 of their paper in *Arch. exper. Path. u. Pharm.*, 1910, Vol. 63).

[2] *Cf.* the instances cited by Czapek, p. 161. H. Nothmann Zuckerkandl has also observed this effect with low concentrations of alcohol and ether (*cf.* footnote 2, p. 317).

[3] Personal communication from Professor J. M. Macfarlane, of the University of Pennsylvania.

[4] *Cf.* Claude Bernard, "Lecons sur les phénomènes de la vie communs aux animaux et végétaux," Paris, 1878. Anæsthesia of plant-growth was also studied by Bernard.

[5] J. Loeb, *Biochem. Zeitschr.*, 1909, Vol. 23, p. 93.

---

[1] *Cf.* Hamburger: *loc. cit.*

[2] *Cf.* H. Nothmann-Zuckerkandl: *Biochem. Zeitschr.*, 1912, Vol. 45, p. 412.

[3] *Cf.* (*e. g.*) my observations on anæsthesia of cleavage in sea-urchin eggs, *Journ. Biol. Chem.*, 1914, Vol. 17, p. 121. The development of astral radiations in dividing egg-cells is prevented by etherization, and existing radiations are suppressed: *cf.* E. B. Wilson: Arch. f. Entwicklungsmechanik, 1901, Vol. 13, p. 353.

[4] R. S. Lillie, *Journ. Exper. Zool.*, 1914, Vol. 16, p. 591.

[5] *Cf.* my papers on antagonisms between salts and anæsthetics; *Amer. Journ. Physiol.*, 1912, Vol. 29, p. 372; Vol. 30, p. 1; 1913, Vol. 31, p. 255.

[6] *Cf.* the papers of Warburg: *Zeitschrift f. physiol. Chemie*, 1910, Vol. 69, p. 452, and Vol. 70, 1911, p. 413; *Pflüger's Arch.*, 1914, Vol. 155, p. 547; Warburg and Wiesel, 1912, Vol. 144, p. 472; Usui, *ibid.*, 1912, Vol. 147, p. 100; Meyerhof, *Pflüger's Archiv*, 1914, Vol. 157, p. 251. Claude Bernard describes the reversible inhibition of yeast-fermentation by anæsthetics (*cf.* footnote 9, below).

[7] E. N. Harvey, BIOLOGICAL BULLETIN, 1915, Vol. 29, p. 308.

[8] *Cf.* Claude Bernard, *loc. cit.*; Overton, "Studien über die Narkose," p. 182.

[9] *Loc. cit.* In Bernard's address, "La Sensibilité," given in 1876 before the French Association for the Advancement of Science, and published in his book, "La Science Expérimentale," Paris, 1890, he cites instances of anæsthesia of the most various vital processes, including photosynthesis, germination and growth in plants, fermentation by yeast, development of the hen's egg,—and concludes: "We may say that everything living is sensitive and can be anæsthetized; whatever is not sensitive is not living and cannot be anæsthetized "(p. 224).

[10] R. S. Lillie, *Journ. Biol. Chem.*, 1914, Vol. 17, p. 121.

In any complete theoretical discussion of anæsthesia it is necessary first to consider the chief conditions under which living cells in general undergo reversible decrease or loss of irritability. This change occurs under a variety of external conditions, mechanical, thermal, electrical and chemical. Mechanical shock may cause temporary loss of irritability. This is probably an effect of over-stimulation and due to prolongation of the refractory period; it resembles in some respects the effect produced in voluntary muscle by poisons like veratrin, which greatly prolongs the relaxation phase and the recovery of irritability following contraction. The paralysis due to mechanical shock differs however from that of anæsthesia in important respects; it represents an injury from which the cell can recover, while true unmixed anæsthesia is quite without injurious action. Certain effects of altered temperature have a closer resemblance to anæsthesia. Most cells and tissues, within the range of temperature in which they show normal activity, show decreased automatic activity with decrease of temperature. Thus according to Snyder[1] the heart of the tortoise shows eighteen beats per minute at 20° and thirty-five at 30°. Observations on the hearts of other animals have given similar results.[2] Within the physiological range of temperature the rate is doubled or trebled by a rise of 10°. This rate of change of velocity with temperature, or temperature-coefficient, is characteristic of chemical reactions in general and is not a distinctively physiological phenomenon. Metabolic and hence vital activity is slowed by cooling just as any other chemical process is slowed. The same temperature-coefficient is shown by a large number of physiological processes including cell-division, rate of conduction in nerve, enzyme action and many others.[3] Thus the above effect of cold is dependent simply on a slowing of chemical processes in cells and has in it nothing distinctively vital. It is important, however, to consider this effect in relation to the problem of anæsthesia, for a simple decrease in reaction-velocity, due to the presence of anticatalytic substances, is held by various investigators to be the essential condition of anæsthesia. Decrease in the rate of a physiological process, like the heart beat, or muscular contraction, or the spread of the excitation-wave in nerve, is not however necessarily associated with a change in the irritability and other vital properties of the tissue; in fact moderate cooling may increase the irritability of nerve. Irritability and rate of metabolic processes represent in fact two independent variables. We infer that anæsthesia is not simply an expression of a decrease in the velocity of certain chemical reactions, such as oxidations, but that some other factor enters, probably physical in nature. Certain other effects of temperature bear a closer resemblance to true anæsthesia. Various irritable tissues become reversibly insensitive at temperatures slightly below or above the normal physiological range. Thus the frog's heart shows an accelerated rate with rise of temperature up to 36° or 37°; it then becomes temporarily inactive and insensitive ("heat-standstill"), but resumes beating if the temperature is lowered. Similarly the musculature of tropical medusæ becomes irresponsive at 40° and recovers on lowering the temperature.[1] This condition of reversible heat-paralysis has certain suggestive resemblances to anæsthesia. Cooling may produce a similar loss of sensitivity in cells whose normal temperature is high, as those of tropical marine animals[2] or warm-blooded vertebrates. Sensory nerve endings, musculature, etc., lose sensitivity if cooled sufficiently, and recover on warming. In these effects structural alterations due to modification of the colloids of the cells (as gelation) are probably concerned; and, as will be shown later, there are indications that similar changes form part of the essential basis of true anæsthesia. The fact that changes of temperature may thus alter the irritability of the tissue independently of their influence on reaction-velocity as such, is highly important to the general theory of narcosis; and it appears unfavorable to those theories which refer anæsthesia to a simple change in the rate of chemical processes like oxidation. Recent experiments by Loeb and Wasteneys[3] on sea-urchin eggs illustrate this. They found that during a condition of narcosis sufficient to arrest cell-division completely, the rate of oxidation is lowered by only 10 per cent.;

---

[1] *Cf.* C. R. Stockard, *Archiv f. Entwicklungsmechanik*, 1907, Vol. 23, p. 249; *Anatomical Record*, 1909, Vol. 3, p. 167 ("The Artificial Production of One-eyed Monsters . . . by the Use of Chemicals"); *Amer. Journ. Anat.*, 1910, Vol. 10, p. 369 ("The Influence of Alcohol and other Anæsthetics on Embryonic Development"). Also McClendon ("Physical Chemistry of the Production of One-eyed Monstrosities") in *Amer. Journ. Physiol.*, 1912, Vol. 29, p. 289. Stockard observed the production of these abnormalities first with magnesium salts, later with lipoid-solvent anæsthetics (alcohol, ether, chloroform, chloretone). Developmental defects are also produced in mammals by alcohol (*cf.* Stockard, *Arch. f. Entwicklungsmech.*, 1912, Vol. 35, p. 569; *Amer. Naturalist*, 1913, Vol. 47, p. 641).

---

[1] University of California Publications, Physiology, 1905, Vol. 2, p. 125.

[2] *Cf.* C. D. Snyder, *Amer. Journ. Physiol.*, 1906, Vol. 17, p. 350; *Zeitschr. f. allg. Physiol.*, 1912, Vol. 14, p. 263; Robertson, BIOL. BULL., 1906, Vol. 10, p. 242; C. G. Rogers, *Amer. Journ. Physiol.*, 1911, Vol. 28, p. 81; BIOL. BULL., 1914, Vol. 27, p. 269; Loeb and Ewald, *Biochem. Zeitschr.*, 1910, Vol. 28, p. 340.

[3] For instances *cf.* Snyder, "Temperature-coefficients of Various Physiological Actions," *Amer. Journ. Physiol.*, 1908, Vol. 22, p. 309.

---

[1] *Cf.* E. N. Harvey, Carnegie Institution Publications, No. 132, 1910, p. 32.

[2] *Cf.* A. G. Mayer, "Effects of Temperature upon Tropical Marine Animals," Carnegie Institution Publications, No. 183, 1914, p. 1.

[3] *Journ. Biol. Chem.*, 1913, Vol. 14, p. 517; *Biochem. Zeitschr.*, 1913, Vol. 56, p. 295.

the same effect on the rate of oxidation results from a simple lowering of temperature by 2° to 3°, a change which only slightly retards cell-division. Decrease in the rate of oxidation as such is thus quite insufficient to account for the inhibitory effect. The fact that, *e. g.*, in frogs' muscle a lowering of temperature of 20° (*e. g.*, from 35° to 15°)—which reduces the rate of oxidation to one fifth of its former value—leaves irritability unimpaired, indicates that any explanation of anæsthesia based on simple decrease in reaction-velocity is inadmissible. A similar decrease in the rate of oxidation can be produced by lipoid-solvent anæsthetics only in concentrations which are much higher than those requisite for anæsthesia.

The constant electric current produces in many irritable tissues effects closely resembling true anæsthesia. Many physiological inhibitions may be caused by passing a constant current through the tissue. There is indeed reason to believe that many of the normal inhibitions, *e. g.*, in the neurones of reflex arcs, are electrical in their nature.[1] The anti-stimulating or desensitizing action of the constant current thus deserves careful consideration in any general theory of anæsthesia. As is well known, the action of the current on irritable tissues like nerve and muscle is *polar*; where the current enters the tissue there is decreased irritability, depression, or inhibition (anelectrotonus); where it leaves there is excitation or heightened irritability (catelectrotonus). Thus a nerve becomes inexcitable near the anode when the constant current is passed; under similar conditions the heart is inhibited and voluntary muscle relaxed. The condition is reversible, and in fact constitutes a typical local anæsthesia. The essential basis of the effect appears to be an altered electrical polarization of the cell-surface. Near the anode, where the current enters the cell or irritable element, the normal outer positivity of the semi-permeable plasma-membrane is increased. Apparently this change renders the membrane irresponsive to stimulation. Variations in the electrical polarization of the plasma-membrane are in all probability constantly associated with variations in irritability. The facts of electrotonus show that such changes of polarization may profoundly alter the irritability and automatic activities of the cell. This general conception is of the greatest importance in the theory of anæsthesia, and will be reconsidered later.

---

[1] *Cf.* my discussion of this possibility in *Amer. Journ. Physiol.*, 1913, Vol. 31, pp. 284 *seq.*

The most important instances of anæsthesia are those produced by chemical substances. First it should be noted that substances belonging to the most various classes may have anæsthetic effects. This fact is overlooked in theories like those of Overton and Meyer, Traube, and others, which refer anæsthesia to the special properties of lipoid-solvent substances, which are regarded as acting either by dissolving in the lipoid constituents of the cell or by adsorption at the surfaces of membranes or other structures. The anæsthetic influence of certain neutral salts shows, however, that lipoid-solubility or surface activity is not essential to narcotic action; magnesium sulphate has long been used by naturalists to narcotize marine animals; more recently it has been applied by Meltzer to produce spinal anæsthesia in mammals. Similar reversible depressant effects are produced by potassium salts. Salts of calcium and strontium also cause reversible desensitization of isolated nerve and muscle. In most animals the calcium-content of the medium has marked influence on irritability and automatic activity; this is well shown in the case of vertebrate muscle; lowering the ratio of calcium to sodium in indifferent media like Ringer's solution has a sensitizing effect, and if the calcium falls too low the muscle twitches spontaneously; increasing the calcium-sodium ratio has a desensitizing action; these effects are reversible.[1] Calcium also antagonizes the stimulating and sensitizing action of pure solutions of sodium and other salts on muscle and nerve. Similarly the heart beats best in media of a certain calcium-content. In marine medusæ (*Rhizostoma* according to Bethe) the rhythmical beat ceases when the animal is transferred to calcium-free sea water, and is restored if calcium is added; still further addition of calcium again arrests the movement.[2] These facts make it clear that alteration of the salt-content of the media may have effects essentially identical with anæsthesia. This is a fact of much theoretical interest, since it indicates that the general condition of the colloids of the cell, especially of the surface-layer or plasma-membrane, is a chief factor in determining the irritability and automatic activity of the living cell. Further evidence of this will be given later. Modification of the properties of this layer may result from an alteration in the state of either its lipoid or its protein constituents, and if this alteration is reversible a temporary inhibition, or anæsthesia, may result. A related condition is seen in the irritable tissues of higher animals, such as muscle and nerve. In these tissues irritability depends on the presence of certain salts in the media; simple withdrawal of salts and replacement by indifferent non-electrolytes like sugar is followed by a temporary loss of irritability; the latter is restored by return to media containing salts, especially sodium salts.[1] The musculature of marine animals (*e. g.*, *Arenicola* larvæ) is similarly inactivated in isotonic solutions of non-electrolytes, and regains irritability in isotonic solutions of various neutral salts. Solutions of sodium salts, together with a small proportion of calcium, are especially favorable. Sodium may be partly replaced by lithium, but not by other metals.[2] Thus the presence of certain salts in the medium is necessary for normal irritability,—hence the effects of isotonic sugar solution, which are due to the absence of salts, not to any special action of the non-electrolyte. The salt-content of the medium may be reduced to a small fraction—one tenth or less—of the normal by diluting the physiological salt solution with isotonic sugar solution, without causing loss of irritability. But with the complete withdrawal of salts irritability soon disappears. In cases like this, where normal irritability is *dependent* on the salt-content of the media, modification of the latter may induce a reversible desensitization closely resembling anæsthesia. Probably several factors enter in the production of this effect, of which the two chief are, a direct change in the properties of the plasma-membrane (colloidal consistency, electrical polarization), and a lowering of the electrical conductivity of the medium.

---

[1] For instances of these various effects *cf.* J. Loeb's article on "Physiological Actions of Ions," in Oppenheimer's "Handbuch der Biochemie," 1909, Vol. 2, p. 104.

[2] *Cf.* Bethe, *Pflüger's Archiv*, 1909, Vol. 124, p. 561.

The reaction of the medium (H-ion concentration) also has profound influence on the irritability and automatic activity of many cells; and a reversible suspension of function akin to anæsthesia may result from a slight change in this reaction. In higher vertebrates the normal reaction of the blood plasma is not far from neutral, and varies only slightly from a constant normal value ($C_H = 0.35 \times 10^{-7}$ to 0.5×10^{-7}); but certain cells of the central nervous system are especially sensitive to such variations. The activity of the respiratory center is apparently regulated by the variations in the H-ion concentration of the blood, cessation of activity resulting from a slight decrease (*i. e.*, increased alkalinity), and increased activity from a slight increase.[1] Reversible cessation of activity may thus result from a slight change in reaction, due, *e. g.*, to loss of CO_2. Similar conditions are known to exist in certain marine animals; thus according to Bethe,[2] slight increase in the alkalinity of the sea water arrests, while slight acidulation accelerates, the rhythmical contraction of medusæ. On the other hand, the activity of many cells and tissues is favored by slight increase in external alkalinity, and depressed by slight acidulation. The irritability and automaticity of living cells are thus largely a function of the reaction of the medium, and this fact has an intimate bearing on the question of the mechanism of anæsthetic and other inhibitions. The precise physico-chemical basis of this action is uncertain, but it probably depends chiefly on alterations in the electrical polarization of the cell-surface. Slight variations in alkalinity or acidity are known to produce marked effects on the electrical polarization of surfaces bathed by media of approximately neutral reaction.[3]

---

[1] *Cf.* Overton, *Pflüger's Archiv*, 1902, Vol. 92, p. 346, and 1904, Vol. 105, p. 176.

[2] R. S. Lillie, *Amer. Journ. Physiol.*, 1909, Vol. 24, p. 459.

The chief chemical substances exerting a reversible depressant influence on a wide range of vital activities are those numerous and chemically diverse organic compounds of which the most evident common property is a solvent action on, or solubility in, fats and fat-solvents. Substances of this class form the majority of anæsthetics in common use; they include alcohols, ethers, esters, aldehydes, ketones, nitriles, amides, various normal and substituted hydrocarbons (chloroform, benzol, etc.) and other related compounds. Most of these bodies are members of homologous series; and it is highly characteristic of such series that the ratio of oil-solubility to water-solubility (oil-water partition-coefficient) increases regularly with increase in molecular weight. At the same time the narcotizing power increases; *i. e.*, in any single series (*e. g.*, alcohols) the higher the molecular weight the lower the concentration required for narcosis. It was this general parallelism that led Overton and Meyer to the view that anæsthetic power, in the case of any substance, is a direct function of its solubility in the fat-like or lipoid constituents of the cell. That a connection exists between the fat-solvent and the anæsthetic properties of a compound had previously been suggested by Bibra and Harless in 1847, and the same view was later expressed by Hermann, C. Bernard, Richet, Ehrlich and others.[1] The first systematic studies of this relationship were however those of Overton and Meyer, the results of whose experiments, carried on independently, were published about the same time (1899).

---

[1] For a recent review and discussion of the evidence *cf.* Winterstein, *Biochem. Zeitschr.*, 1915, Vol. 70, p. 45.

[2] *Pflüger's Archiv*, 1909, Vol. 127, p. 219.

[3] *Cf.* Haber and Klemensiewicz, *Zeitschr. f. physik. Chemie*, 1909, Vol. 67, p. 385.

In a study of the permeability of animal and plant cells to various types of compounds, Overton[2] had reached the conclusion that solubility in lipoids was the chief factor determining the ready entrance of a compound into cells; compounds with well-marked power of penetration belonged chiefly to the narcotic group; and in a later extensive investigation on narcosis in tadpoles[3] a far-reaching parallelism was found between the oil-water partition-coefficients of a wide range of organic compounds and their narcotizing action. The nature of Overton's results may be best seen from the following series, which gives the concentrations required to narcotize tadpoles in the case of the ethyl esters of the first five fatty acids. (See Table I.)

The narcotic action is seen to increase steadily with decrease in the water-solubility,—*i. e.*, increase in the ratio of partition between oil and water. Each member of the series is from two to three times as effective as its immediate predecessor. This rule appears to hold very generally for members of homologous series, and a large number of similar instances have been collected by Traube and other recent investigators.[1] Numerous other experiments with alcohols, hydrocarbons, aldehydes, ketones, etc., showed a similar increase in narcotic action with increase in the oil-water partition-coefficients. Overton accordingly drew the conclusion that narcotics act by *dissolving* in certain substances, contained especially in nerve-cells, which resemble fats in their solvent properties; these substances are the lipoids, especially lecithin and cholesterin, which appear to be essential constituents of protoplasm; it is the *physical* modification of these substances, due to their being charged or impregnated with the lipoid-soluble narcotic, that forms the essential condition of anæsthesia. Meyer's conclusion was similar;[2] the narcotizability of cells is thus related to the nature and the proportion of the lipoids present in the protoplasm; the high susceptibility of nerve-cells is probably dependent on their high lipoid-content. The unequal action of different narcotics depends on their unequal partition-coefficients, which determine their distribution in a mixture of water and lipoid substances. The greater the relative lipoid-solubility the larger the proportion of the anæsthetic present in solution in the lipoid cell-constituents when the partition-equilibrium is reached. Hence, if the lipoid-solubility of a substance is very high, extremely dilute solutions may exert anæsthetic action. Overton, for example, found that phenanthrene could narcotize tadpoles in dilutions so low as one part in 1,500,000 of water.

---

[1] For an account of these earlier views *cf.* Overton, "Studien über die Narkose," June, 1901.

[2] Overton, "On the General Osmotic Properties of Cells," etc., *Vierteljahrsschr. d. naturf. Gesellsch. in Zürich*, 1899, Vol. 44, p. 88.

[3] "Studien über die Narkose," 1901.

TABLE I.

Ester.	Narcotizing Concentration. (Mols per Liter).	Solubility in Oil and Water.
Ethyl formate	0.07*m*–.09*m*	Oil: water = 4 : 1
" acetate	.03*m*	In 15.2 parts water; in all parts oil
" propionate	.01*m*–.012*m*	" 50 " " " " " "
" butyrate	.0043*m*	" 190 " " " " " "
(" isobutyrate)	.0057*m*	" 140 " " " " " "
" valerianate	.0019*m*	" 500 " " " " " "

Overton's study of permeability had led him to the conclusion that the outer layer or plasma-membrane of cells consists largely of lipoid material; in this way he explained the ready entrance of lipoid-soluble substances into cells. Now it is an evident corollary of Overton's hypothesis that if the anæsthetic acts by changing the physical state of the lipoid cell-constituents it must affect the properties of a lipoid-rich cell-structure like the plasma-membrane. Overton, however, does not refer narcotic action specifically to a modification of the plasma-membrane alone, but to a general modification in the physical state of all cell-lipoids, wherever situated. Recently, however, much evidence has accumulated indicating that the essential influence is that exerted on the plasma-membrane, and that it is the modification in the properties of this structure which determines the characteristic anæsthetic effect. This evidence and its implications will be considered later.

---

[1] *Cf.* Traube, "Theorie der Narkose," *Pflüger's Archiv*, 1913, Vol. 153, p. 276.

[2] Hans Meyer, *Arch. f. exper. Path. u. Pharm.*, 1899, Vol. 42, p. 109.

The hypothesis of Overton and Meyer has received wide acceptance. It is not clear, however, why simple solution of chemically indifferent substances in the lipoids of the tissue should so modify its irritability; and Overton and Meyer do not attempt to explain this connection. The parallelism between lipoid-solubility and narcotic action is not an exact one, and many exceptions to the rule are known. The powerful narcotic action of chloral hydrate, which is several times more soluble in water than in oil, is not thus explained; and lipoid-insoluble neutral salts of magnesium and other metals may exert typical narcotic action. Evidently other factors than solubility may enter. Yet the evidence adduced by Overton and Meyer, as well as by more recent investigators, leaves no doubt that in the case of organic anæsthetics high lipoid-solubility is typically associated with marked narcotic action. The reversibility of anæsthesia corresponds to the reversibility of the process of solution. The chemical indifference of many anæsthetics is thus not surprising, since the substance acts not by chemical combination but by simple solution in the cell-lipoids.

According to Overton and Meyer's hypothesis it is this *solution* of the narcotic in the lipoid which determines anæsthetic action. This view has recently been attacked from various sides. According to Traube,[1] the anæsthetic acts not by *dissolving* in the cell lipoids, but rather by undergoing surface-condensation or adsorption at the physiologically active surfaces within the living system; these may be the surfaces of special cell-structures or of colloidal particles, whether lipoid or protein. The catalytic activity of these surfaces is thus decreased, and the reaction-velocities of essential chemical processes, especially oxidations, is lowered. A corresponding depression of cell-functions results. Whether this effect is to be attributed to a displacement of metabolically active water-soluble substances like sugar, whose surface-activity is relatively small, or to a direct alteration in the catalytic properties of the physiologically active surfaces themselves, is uncertain. The essential feature of Traube's view is that it regards the *surface-activity* of a narcotic compound, *i. e.*, its influence in lowering surface-tension—rather than its lipoid solubility—as the determining factor in its depressant action. This surface-activity determines the degree of adsorption, and hence, indirectly, of anæsthetic action. It is well known that the surface-tension of such a solvent as water, in contact with air or with another liquid or a solid, is greatly influenced by the presence of dissolved substances. This influence is usually in the direction of a decrease. A few substances like inorganic salts and sugars increase the surface-tension of water, although the effect is slight; but the majority, especially of organic substances, cause well-marked and often great decrease. This is especially true of substances whose water-solubility is limited; and in general the more soluble a substance is in oils or other water-insoluble organic solvents, and the less soluble in water, the greater is its influence (for a given molecular concentration) on the surface-tension of water. In a capillary tube the level of pure water or of an aqueous solution is raised, by the contractile force or tension of the surface of the water-film lining the walls of the tube, to a certain height above the level of the water outside. This height (h) is proportional to the surface tension of the water (σ), and inversely proportional to the radius of the tube (r) and the specific gravity (g) of the liquid ($h = 2\sigma/rg$). The relative surface-tensions of aqueous solutions may thus be determined by measuring the heights to which the column of solution is raised by capillarity in a given tube. This height is decreased by surface-active substances, and the degree of capillary activity or tension-lowering action of different substances can thus be determined. Now in any homologous series this action (for equimolecular solutions) is found to decrease as the molecular weight increases. The surface-tension in milligrams per linear centimeter (*i. e.*, the pull exerted by a strip of surface one centimeter wide) of $m/4$ solutions for the first five aliphatic alcohols at 15° is given by Traube as follows: the surface-tension of the $m/4$ solution of dextrose, a physiologically important surface-inactive compound, is given for comparison.

---

[1] I. Traube, "Theorie der Narkose," *Pflüger's Archiv*, 1913, Vol. 153, p. 276, and Vol. 160, 1915, p. 501; also "Theorie des Haftdrucks und Lipoidtheorie," *Biochem-Zeitschr.*, 1913, Vol. 54, p. 305, and other papers cited there.

TABLE II.

Liquid (Solutions = $m/4$).	Surface-tension (Milligrams per Centimeter).	Molecular Concentrations of Isocapillary Solutions.	Concentration for Narcosis of Tadpoles (Overton).
Water	73		
$m/4$ dextrose	73.3		
Methyl alcohol	70.5	14.0	0.52m–0.62m
Ethyl "	67.3	5.0	0.27m–0.31m
n-propyl "	58.9	1.6	0.11m
i-butyl "	44.9	0.46	0.045m
i-amyl "	30.5	0.14	0.023m

The second column gives the concentrations required to effect a definite lowering of surface-tension. It will be observed that the surface-activity of each member (as measured by the reciprocals of the isocapillary concentrations) is approximately a third of that of its immediate successor. The third column shows that the narcotic activity increases from each member to the next following in a closely similar proportion. Results of this kind are on the whole typical for homologous series. The question arises as to their general physiological significance.

According to Traube the essential physico-chemical factor in these physiological effects is the characteristic influence which surface-tension has upon the distribution of dissolved substances in any polyphasic system. The general principle of Willard Gibbs and J. J. Thomson states that substances which lower the surface-tension of any solvent attain, when equilibrium is reached, a higher concentration in the surface-layer than in the interior of the solvent; a surface-condensation or adsorption thus results, which is the greater the greater the surface-activity of the dissolved compound. Hence substances having a high degree of surface-activity are as a class readily adsorbed. The effect is the same as if a relatively slight coherence existed between the solvent and the dissolved substance. Hence Traube conceives of a surface-active substance as one in which the union or adhesion between solute and solvent is slight; *i. e.*, relatively little work is required to separate the substance from solution; and he has introduced the expression "Haftdruck" (adhesion-tension or solution-affinity) to designate this condition. The capillary activity of any substance in a given solvent varies inversely with its solution-affinity (Haftdruck) relatively to that solvent. The lower the solution-affinity relatively to water the greater is the tendency of any substance to pass out of its solution in water; this tendency favors the entrance of capillary-active substances into other adjoining solvents or media, *e. g.*, into and through the membranes bounding cells.[1] The ready penetration of such substances into living cells is in fact referred by Traube not to lipoid-solubility, but to low solution-affinity in relation to the medium bathing the cell. A tendency to surface-condensation or adsorption is a characteristic accompaniment of low solution-affinity to water; the marked physiological activity shown by surface-active substances as a class is a direct consequence of this tendency.

According to data already cited, the narcotic activity of organic substances shows a parallelism with both capillary activity and lipoid-solubility. Other physiological effects (*e. g.*, membrane-formation in sea-urchin eggs,[1] reversal of the sense of heliotropism, sensitizing action, cytolytic action) show a similar parallelism. The question of whether the particular physiological effect under consideration is determined by one or the other factor, or by the interaction of both, has to be decided by further evidence. In favor of the view that lipoid-solubility rather than surface-activity is the essential determining factor in the action of lipoid-soluble narcotics, is the fact that the action varies with temperature in the same direction as the oil-water partition-coefficient. This is shown in a remarkable manner in the following table from Hans Meyer.[2] The concentrations required to narcotize tadpoles were determined at the two temperatures 3° and 30° using (*a*) narcotics whose relative solubility in oil *decreases* with rise in temperature, and (*b*) where it *increases*. The critical anæsthetizing concentrations for the following six anæsthetics are given in the table.

---

[1] Traube's attempts to apply this conception to a general explanation of osmotic phenomena are not convincing. The fact that lipoid-solubility varies in the same direction as capillary activity makes the question difficult to decide by experiments on living cells. But in the case of dead cells, as well as of more permeable partitions like parchment paper or collodion, surface-active and surface-inactive substances appear to penetrate with equal ease. The difference between the rate of penetration of dissolved lipoid-insoluble substances into living and into dead cells shows that the permeability of the partition (*i. e.*, relative resistance to diffusion) is the deciding factor in their entrance. Furthermore, instances are well known where the rate of penetration of a substance through an artificial partition varies directly with its solubility in the material composing the partition. Flusin's experiments with rubber membranes are a good instance of this. The diffusion of substances through the surface-films bounding the cells implies a passage either through or between the membrane-constituents; and in the case of the living cell, solution of lipoid-soluble substances in the lipoids of the membrane is probably the main factor in their entrance, although this entrance may be favored by adsorption due to surface-activity.

Table III.

Anæsthetic.	Critical Concentration for Anæsthesia.		Oil/Water Partition-Coefficients.	
	At 3°.	At 30°.	At 3°.	At 30°.
A. Salicylamide........	*m*/1300	*m*/600	22.23	14
Benzamide.........	*m*/500	*m*/200	0.67	0.43
Monoacetin........	*m*/90	*m*/70	0.099	0.066
B. Ethyl alcohol.......	*m*/3	*m*/7	0.026	0.047
Chloral hydrate.....	*m*/50	*m*/250	0.053	0.236
Acetone...........	*m*/3	*m*/7	0.146	0.235

In the first three compounds the relative lipoid-solubility decreases and the narcotizing concentration increases with rise of temperature; while with the others the conditions are reversed. Thus simple cooling suffices to restore activity to tadpoles anæsthetized in chloral hydrate at 30°. If adsorption under the influence of surface-tension were the main factor in these effects, such a change of narcotic power with temperature would be inexplicable, since surface-tension is influenced in a totally different manner by change of temperature. The fact that anæsthetics collect in cells in higher concentration than in the medium also favors the partition rather than the adsorption theory of narcosis. Chloroform, ether, and esters undergo concentration in nervous and other tissues, as Pohl[1] and Hedin[2] have shown. Warburg and Wiesel[3] have also found in the case of yeast that the tendency of compounds to concentrate in cells increases with increase in their narcotic power. In solutions that diminished fermentative activity by one half, phenyl urethane was found to be three times and thymol nine times more concentrated in the cells than in the medium. These facts strongly suggest a distribution according to relative solubilities.

---

[1] *Cf.* J. Loeb, "Artificial Parthenogenesis and Fertilization," University of Chicago Press, 1913, Chapter 14.

[2] H. Meyer, *Arch. f. exper. Path. u. Pharm.*, 1901, Vol. 46, p. 338.

What Traube especially insists upon is that effects similar to narcosis are shown in cases where lipoid-solubility can play no part. Thus according to Warburg and Wiesel[4] the fermentative and oxidative activities shown by lipoid-free preparations of dried microörganisms are influenced by the lipoid-solvent anæsthetics in the same manner as in the intact organisms. It is to be noted, however, that the effective concentrations are much higher in the case of such preparations than in that of living cells. Traube cites a large number of observations made with solutions of various surface-active substances, showing that with both animal and plant cells, as well as with enzymes, the degree of narcotic and cytolytic action—of inhibition and destruction in the case of enzymes—is nearly proportional to the surface-activity of the solution.[5] Solutions of widely different substances, provided they have the same surface-tension ("isocapillary" solutions), have equal physiological action. In the following table I have collected a number of observations illustrating the various physiological effects produced by members of the aliphatic alcohol series. In each instance the molecular concentrations required to produce a definite physiological effect are given; the molecular concentrations which cause equal lowering of surface-tension (isocapillary concentrations) are given at the end of the table.

---

[1] Pohl, *Arch. f. exper. Path. u. Pharm.*, 1891, Vol. 28, p. 239.

[2] Hedin, *Pflüger's Archiv*, Vol. 68, p. 229.

[3] Warburg and Wiesel, *Pflüger's Archiv*, 1912, Vol. 144, p. 472.

[4] *Loc. cit.*, p. 471.

[5] *Cf.* Traube, "Theorie der Narkose," *loc. cit.*

Table IV.

Physiological Effect.	Concentrations of Alcohol (Mols per Liter) for Producing Effect.							
	Methyl.	Ethyl.	Propyl.	Butyl.	Amyl.	Hexyl.	Heptyl.	Octyl.
Narcosis of tadpoles (Overton)[1]	0.57	0.29	0.11	0.038	0.023	....	....	.0004
Narcosis of *Arenicola* larvæ[2]	2.2	1.1	0.34	0.09	0.03	....	....	.001
Prevention of cleavage in *Arbacia* eggs[3]	....	0.87	0.27	0.086	0.037	....	....	.001
Checking of development in *Strongylocentrotus* eggs (Fühner)[4]	0.72	0.41	0.136	0.045	....	....	0.0017	.0005
Narcosis of *Daphnia* (Loeb)[5]	1.2	0.6	0.2	0.12	0.04	....	....	....
Production of heliotropism *Daphnia* (Loeb)[5]	0.6	0.2	0.05–0.1	.04	....	....	....	....
Haemolysis (Fühner and Neubauer)[6]	7.34	3.24	1.08	0.318	0.091	0.034	0.012	.004
Decreasing oxidations by 50 per cent. in blood-corpuscles (Warburg)[7]	5.0	1.6	0.8	0.15	0.045	....	....	....
Inhibition of fermentation by ether-extracted yeast (Warburg)[8]	5.0	3.5	1.3	0.54	0.23	....	....	....
Depression of isolated tortoise ventricle by 50 per cent. (Vernon)[9]	1.1	0.53	0.23	0.05	0.02	....	....	....
Destruction of indophenol oxidase (Vernon)[10]	10.5–14	4.8–8	1.5–2.75	0.32–0.9	....	....	....	....
Precipitation of nucleo-proteid of liver (Battelli and Stern)[11]	5.7	2.38	1.12	0.45	0.21	....	....	....
Destruction of oxydon of ox-muscle (Battelli and Stern)[11]	7.54	3.57	1.16	0.44	0.19	....	....	....
Decrease in action of yeast invertase (Meyerhof)[12]	3.0	1.3	0.5	0.27	0.21	....	....	....
Concentrations of isocapillary solutions	14.0	5.0	1.6	0.46	0.14	....	....	....

---

[1] Overton, "Studien über die Narkose," p. 101.

[2] R. S. Lillie, *Amer. Journ. Physiol.*, 1912, Vol. 29, p. 372; 1913, Vol. 31, p. 255.

[3] R. S. Lillie, *Journ. Biol. Chem.*, 1914, Vol. 17, p. 121. The concentrations of alcohols preventing the activation of unfertilized *Arbacia* eggs by pure isotonic solutions of KCNS and NaI are similar; *cf. Journ. Exper. Zoology*, 1914, Vol. 16, p. 591 (see table, p. 607).

[4] Fühner, *Zeitschr. f. Biol.*, 1912, Vol. 57, p. 465.

[5] J. Loeb, *Biochem. Zeitschr.*, 1909, Vol. 23, p. 93.

[6] Fühner u. Neubauer, *Arch. f. exper. Path. u. Pharm.*, 1907, Vol. 56, p. 333.

[7] Warburg, *Zeitschr. f. physiol. Chem.*, 1910, Vol. 69, p. 452.

[8] Warburg and Wiesel, *Pflüger's Archiv*, 1912, Vol. 144, p. 472.

[9] H. M. Vernon, *Journ. Physiol.*, 1911, Vol. 43, p. 325.

[10] Vernon, *Journ. Physiol.*, 1912, Vol. 45, p. 197.

[11] Battelli and Stern: *Biochem. Zeitschr.*, 1913, Vol. 52, p. 226.

[12] Meyerhof: *Pflüger's Archiv*, 1914, Vol. 157, p. 251.

These data show that the increase of surface-activity observed on passing from one member of the series to the next is very generally associated with a proportionately similar increase of physiological activity. In general each member has from two to three times the capillary activity of its immediate predecessor; and the same holds true in a general way for its physiological activity. It is also to be noted, however, that in general the same holds true for lipoid-solubility.

If physiological activity is in fact a function of capillary activity, solutions of equal surface-tension ought to exhibit equal narcotic or other physiological effects. Traube cites various observations indicating that this is frequently the case. Thus Czapek[1] has determined for a large number of organic substances the surface-tensions of the solutions which have equal effects in liberating tannin from plant cells (the leaves of *Echeveria*); this effect is analogous to hæmolysis and depends on increase in the permeability of the plasma-membrane. The surface-tensions of equally effective solutions (against air) were found to approach a fairly constant value, about two-thirds of that of pure water. Kisch[2] also found that isocapillary solutions had equal effects in preventing germination of yeast; and H. Zuckerkandl[3] obtained similar results for the inhibition of protoplasmic streaming in plant cells. The results of observations by Führer and Neubauer and also by Traube himself on hæmolysis are similar. Thus, taking again the series of alcohols: the surface-tensions of the least concentrated solutions which free tannin from Echeveria leaves and which inhibit the germination of yeast cells are as follows: (water = 1).

TABLE V.

	Critical Surface-tensions of Solutions Causing	
Alcohol.	*A*. Exomosis of Tannin from *Echeveria* Cells.	*B*. Inhibition of Growth of Yeast.
Methyl	0.7	0.51
Ethyl	0.67	0.48
n-propyl	0.675	ca. 0.49
i-propyl	0.69	——
n-butyl	0.69	——
i-butyl	0.665	ca. 0.495
i-amyl	0.665	0.49

---

[1] *Cf.* Czapek, "Über eine Methode zur direkten Bestimmung der Oberflächenspannung der Plasmahaut von Pflanzenzellen," Jena, G. Fischer, 1911.

[2] Kisch, *Biochem. Zeitschr.*, 1912, Vol. 40, p. 152.

[3] H. Nothmann-Zuckerkandl, *Biochem. Zeitschr.*, 1912, Vol. 45, p. 412.

In each instance equal physiological effects are produced by solutions of approximately equal surface-tension. Czapek finds that the same rule of equal action for isocapillary solutions holds good for ketones, esters, urethanes, and other compounds. Lately, however, Vernon,[1] Höber,[2] and others have pointed out various exceptions to this rule; thus chloroform, chloral hydrate, nitromethane, and ethylene glycol begin to set free tannin in solutions of much higher surface-tensions than the above. And with the higher alcohols the surface-tensions of the effective solutions are lower than the theory requires. These deviations from the rule of isocapillarity are referred by Traube partly to chemical influences (*e. g.*, acid in chloral hydrate solutions), partly to incorrect determination of surface-tension in solutions of volatile substances like chloroform and ether, partly to the influence of viscosity. In general it appears that the more viscous compounds, *e. g.*, the higher alcohols, show equal physiological action, *e. g.*, hæmolysis, in solutions of lower surface-tension than Traube's theory demands; Traube, however, believes that this difference is due simply to slowness of penetration, incident to the high viscosity of the adsorbed layer of the narcotic at the surfaces (*e. g.*, of red corpuscles) where the essential action takes place. For solutions of approximately equal viscosity the rule of equal action with equal capillarity appears to hold true. In general isocapillary solutions of surface-active substances have less hæmolytic or other physiological action the greater their viscosity; hence equal action for isocapillary solutions is to be expected only when the viscosities are similar.[3] Even this, however, is not always the case. For instance, Loeb[4] has found that weak solutions of fatty acids, as well as weak solutions of narcotics, produce positive heliotropism in daphnids; the least effective concentrations for the first six members of the acid series were: .006*n* formic, .006*n* acetic, .005*n* propionic, .004*n* butyric, .004*n* valerianic, .002*n* caproic. The increase in effectiveness with increasing molecular weight is much more gradual than the increase in capillary activity. The influence of the H-ion concentration enters here, and probably constitutes the preponderant factor. The part played by purely chemical action seems to be underestimated in Traube's theory. Where this factor enters, surface-activity may be of subordinate importance. Thus in the case of neutral salts solutions of equal surface-tensions may have entirely different action on colloids and hence on living cells. Traube's rule is at best an approximation; but its significance is not to be underestimated on this account. Adsorption and surface-condensation undoubtedly run parallel with capillary activity; this is a matter not only of deduction from the Gibbs-Thomson principle, but also of direct observation; and surface-forces play so large a part in biological processes that it is not surprising to find a frequent parallelism between the physiological effects of solutions and their surface-activity.

---

[1] Vernon, *Biochem. Zeitschr.*, 1913, Vol. 51, p. 1.

[2] Rudolf Höber, "Physikalische Chemie der Zelle und der Gewebe," 4th edition, 1914, pp. 415 *seq.*

[3] *Cf.* Traube, "Influence of Viscosity and Surface-tension in Biological Processes," *Internat. Zeitschr. f. physik-chem. Biol.*, 1914, Vol. 1, p. 275.

[4] J. Loeb, *Biochem. Zeitschr.*, 1909, Vol. 23, p. 95.

Traube in fact recognizes that the lipoid-content of cells may have an influence on the rapidity of intake of the narcotic (since lipoid-solubility will naturally favor penetration), and hence may be a factor in the narcotic action; but the narcosis itself does not depend on this solution in lipoids; "the lipoid-content influences narcotic action but does not determine it; even lipoid-free cells may be narcotized."[1] This conception, however, does not seem adequate in view of the observations of Meyer cited above, on variation of narcotic action with temperature.

It is important to note that surface-active substances affect not only biological processes but also catalyses of various kinds, especially those due to enzymes and other colloidal catalyzers (platinum, etc.), where the action is probably dependent on the character and extent of the surface between catalytic agent and medium (heterogeneous catalyses). Enzymes are colloidal catalyzers formed in cell-metabolism; as colloids it is to be expected that surface-conditions will largely influence their action. Recently Bayliss[2] has emphasized the importance of such conditions.

[1] "Theorie der Narkose," *loc. cit.*, p. 305.

[2] *Cf.* Bayliss, "The Physiological Importance of Phase-boundaries," *Science*, N. S., 1915, Vol. 42, p. 509. For the influence of anæsthetics on invertase and on colloidal platinum, *cf.* Meyerhof, *Pflüger's Archiv*, 1914, Vol. 157, pp. 251, 307; *cf.* also Warburg, *ibid.*, Vol. 155, p. 547, for their influence on the catalytic activity of finely divided carbon (animal charcoal).

He has shown that various enzymes (lipase, emulsin, urease, trypsin) retain their activity when suspended in media in which they are insoluble. This fact is best explained on the view that increased concentration of the substrate at the surface of the enzyme particles is mainly responsible for the increased reaction-velocity in its presence. This view does not explain specificity, which is probably a matter in which selective adsorption and stereochemical conditions enter as factors; but it leads to the general expectation that readily adsorbable, *i. e.*, surface-active, substances will as a class have marked influence on enzyme action.

In many organisms oxidations are the chemical processes which are most evidently influenced by narcotics; and the view that narcotic action consists essentially in a suppression of intracellular oxidations has gained wide favor, and has been supported chiefly by Verworn in Germany, and by Mathews, Loevenhart and others in America. The view that this anti-oxidative action may be exerted directly upon the oxygen-catalyzers of the cell is supported by Traube. Considerable evidence consists favorable to this view. Thus Warburg finds that inorganic iron salts accelerate oxidations in disintegrated sea-urchin eggs, and he further finds that this accelerative action is checked by urethane.[1] This interesting observation suggests the possibility that catalysis by iron plays a part in intracellular oxidations; this catalysis is checked by anæsthetics, and it is to be inferred that oxidative processes under the influence of organic catalyzers or oxidases would be similarly affected. In support of this conception Traube cites various instances where oxidative processes are checked by surface-active or narcotic substances.[2] Such instances include the decomposition of hydrogen peroxide by platinum (Bredig), the oxidation of sodium sulphite by free oxygen (Bigelow, Titoff, Young; Young finds this process checked by traces of morphine, brucine, nicotine, and especially quinine; and Traube even refers the antipyretic action of quinine to its inhibiting action on oxidation); the oxidation of phosphorus and phosphorus trioxide by free oxygen (Centnerszwer, Scharff); oxidation of stannous chloride (Young); oxidation by tissue-oxidases (Vernon, Baer and Meyerstein); catalytic oxidation of oxalic acid by animal charcoal (Warburg).[1] Thus not only oxidations under the influence of heterogeneous or colloidal catalyzers may be checked by surface-active substances, but also oxidations in homogeneous solution. This would indicate that surface-activity is not the only factor involved. On the basis of this and other facts Traube puts forward the hypothesis that narcotics are essentially negative catalyzers, especially in relation to oxidative processes.[2] The question of how this anti-oxidative effect is produced within the living cell is the essential one. Traube and others have suggested that the direct action of surface-active and narcotic substances on colloids may be a chief factor. Moore and Roaf[3] have investigated the precipitation of serum by such substances, an effect which shows a general increase with surface-activity. These authors, however, refer the effect to the formation of loose chemical combinations between the narcotic and the proteins; the quantity of chloroform and other anæsthetics dissolved by serum is several times greater than by water; they regard the excess as held by chemical union, and they attribute narcotic action to such loose protein-anæsthetic compounds which limit the chemical activities of the protoplasm, including presumably the oxidations. Warburg and Wiesel also find that narcotic substances have a precipitating action on the press-juice of yeast, and that the anti-fermentative action runs parallel with the precipitating action; and they recall the older view of Claude Bernard, according to which a semi-coagulation of the cell-colloids forms the basis of narcosis.[4] Battelli and Stern[5] find that the nucleo-proteins of cell-extracts are also precipitated by lipoid-solvent anæsthetics, and that the precipitating action runs parallel with the influence in checking the activity of cell-oxidases or oxydones. Traube also cites observations by Schryver[1] in support of this general point of view; surface-active substances retard the gelation of certain colloidal solutions, *e. g.*, of sodium cholate under the influence of calcium salts, and the degree of retardation runs parallel to capillary activity and narcotic action. Physical alterations of the cell-celloids may thus lie at the basis of the anti-oxidative action which, according to this view, conditions the narcotic action. Other instances of this effect will be considered later. Traube expresses his essential view as follows: "the physical alterations of the cell-colloids—and by no means of the lipoids alone—form one of the most essential conditions for the slowing of chemical processes in cells, and hence also for narcotic and other toxicological processes. These physical alterations are a consequence of the depressant influence which narcotics exert upon surface-tension, and upon the internal pressure of the cell contents."[2] According to this conception the physical alteration of the colloids would be the primary effect of the narcotic, and decrease of oxidation secondary; this view is more consistent with the membrane-theory of narcosis, about to be described, than with the previously quoted view which refers narcosis to a direct anti-catalytic action. According to the membrane-theory, it is the plasma-membrane which is primarily affected; and the decrease of oxidations (when this occurs) is a secondary consequence of the change in the membrane. This view would make the direct action of the anæsthetic on oxidation-processes relatively unimportant. To regard anæsthesia as dependent on a direct anti-catalytic action seems insufficient, especially in view of the fact that the effective anti-catalytic concentrations are much higher than those required for narcosis. It should also be remembered that magnesium sulphate and other salts can act as anæsthetics—such salts can have no such direct anti-catalytic action—also that the electric current may show the same influence. The action of anæsthetics on oxidases will shortly be considered in more detail.

---

[1] Warburg, *Zeitschr. f. physiol. Chem.*, 1914, Vol. 92, p. 231.

[2] Traube, "Über Katalyse," *Pflüger's Archiv*, 1913, Vol. 153, p. 309.

---

[1] Warburg, *Pflüger's Archiv*, 1914, Vol. 155, p. 547.

[2] Winterstein ("Heat-paralysis and Narcosis," *Zeitschr. f. allg. Physiol.*, 1905, Vol. 5, p. 323) had earlier compared narcotics to the anticatalyzers or "Paralysatoren" of Bredig.

[3] Moore and Roaf, *Proc. Roy. Soc.*, 1904, Vol. 73, p. 382; 1906, Vol. 77*B*, p. 86.

[4] *Cf.* Warburg and Wiesel, *loc. cit.*; also Claude Bernard, "Leçons sur les anesthésiques et sur l'asphyxie," Paris, 1875, p. 154. Anæsthetics, however, do not affect the osmotic pressure of protein solutions, according to Meyerhof (see footnote 1, p. 346).

[5] *Loc. cit.*

The general fact that surface-active substances alter the physical condition or state of aggregation of many colloids is, however, highly important in any general theory of anæsthesia.

---

[1] Schryver, *Proc. Roy. Soc., B*, 1914, Vol. 87, p. 366.

[2] "Theorie der Narkose," p. 302.

Probably this effect is to be related to their influence on the electrical potential difference normally existing between the colloidal particles and the medium. Gouy[1] found that the potential-difference between mercury and sulphuric acid in the capillary electrometer is lowered by the presence of many surface-active or narcotic substances; similar observations were made by Abl[2] for cadmium amalgam cells, and by Grumbach[3] for various contact-potentials; and according to Traube the order of relative action in all of these cases is essentially that of capillary activity. Now precipitation or increased aggregation of colloids is typically associated with decrease in the electrical polarization of the colloidal particles; and capillary-active substances which produce this latter effect ought therefore to further such precipitation. A similar influence of anæsthetics on the potentials shown by organic membranes like apple-skin against salt solutions was observed by Loeb and Beutner;[4] the concentrations required for appreciable lowering of potentials were, however, much higher than those ordinarily required for anæsthesia. Notwithstanding this difficulty Traube suggests that a decrease in contact-potentials, as well as of surface-tension at the active surfaces in tissues like nerve, may be an important factor in the action of narcotics. To quote from Traube's recent paper on narcosis: "The narcotic substances, in collecting at the boundary-surfaces of cell-wall and cell-fluid, lower there the electrical contact-potentials, and in so doing directly prevent the transmission of motor and sensory impulses by means of nerve-centers. . . . This retarding or inhibiting action, exerted by substances of low solution-affinity (Haftdruck) to water, upon the oxidations and other intracellular processes conditioned by cell-colloids, and also upon the electrical phenomena at boundary-surfaces, is the cause of that condition which we designate as narcosis."[5] A somewhat similar view had previously been expressed by A. B. Macallum: "Chloroform, ether, alcohol, and chloral lower surface-tension in aqueous solutions, in blood plasma and lymph, and in all probability also the surface-tension of all cells, but especially of the nerve-cells. This would make them incapable of receiving or transmitting a nerve-impulse."[1]

---

[1] Gouy, *Annales de chimie et de physique*, 1906, Sér. 8, Vol. 8, p. 291, and Vol. 9, p. 75.

[2] Abl, Dissertation; Bonn, 1907 (cited from Traube, *Pflüger's Archiv*, 1910, Vol. 132, p. 521).

[3] Grumbach, *Annales de chimie et de Physique* (8), 1911, Vol. 24, p. 463.

[4] Loeb and Beutner, *Biochem. Zeitschr.*, 1913, Vol. 51, p. 303.

[5] "Theorie der Narkose," p. 306.

The general view that narcosis is essentially a phenomenon of asphyxia or retarded oxidation is an old one, suggested by Claude Bernard and others, and has been revived in somewhat different form of recent years, chiefly through the influence of Verworn[2] and his pupils. Decrease in oxidations is in fact frequently observed during narcosis. Thus Alexander and Cserna[3] showed by direct analysis of blood a marked decrease in the oxygen-consumption of the brain during ether and morphine narcosis; oxidation-processes in the liver are also decreased under the influence of various narcotics.[4] But whether this decrease is simply a consequence of a paralysis of metabolic as well as of other functions, or whether it is the primary and determinative condition, is a question which has been answered differently by different investigators. Verworn has identified narcosis with asphyxia chiefly because of certain similarities between the physiological behavior of asphyxiated and narcotized tissues and cells. A summation of the effects of narcosis and of asphyxia is seen under certain conditions; thus Winterstein found that frogs asphyxiated by perfusion with oxygen-free salt solution until reflex activity was lost showed no recovery from the asphyxia if supplied with oxygen while still in a state of anæsthesia.[5] The condition of narcosis appears to render oxygen unavailable to the cells. Fröhlich[6] found the same rule to hold for nerve-trunks; normally oxygen revives irritability which has been lost in an oxygen-free medium (nitrogen atmosphere);

---

[1] A. B. Macallum, "Surface-tension and Vital Phenomena," Univ. of Toronto Studies, 1912, No. 8, p. 70.

[2] *Cf.* Verworn, "Die Narkose," Jena, 1913; *cf.* also Harvey Lectures, 1911–12, p. 52. Cl. Bernard ("Leçons sur les anesthésiques et sur l'asphyxie," pp. 92 *seq.*) discusses the question whether anæsthesia is a form of asphyxia, but rejects this view on the ground that anæsthetized animals show no signs of general asphyxia (p. 97).

[3] Alexander and Cserna, *Biochem. Zeitschr.*, 1913, Vol. 53, p. 100.

[4] *Cf.* Joannovics and Pick, *Pflüger's Archiv*, 1911, Vol. 140, p. 327; Baer and Meyerstein, *Arch. f. exper. Path. u. Pharm.*, 1910, Vol. 63, p. 441.

[5] Winterstein, *Zeitschr. f. allg. Physiol.*, 1902, Vol. 1, p. 19.

[6] Fröhlich, *Zeitschr. f. allg. Physiol.*, 1904, Vol. 3, p. 75.

but oxygen has no such effects on narcotized nerves; similar observations were made by Nagai[1] on ciliated epithelium. There is thus no recovery from asphyxia during narcosis, even with a good supply of oxygen. Nerves subjected to prolonged narcosis show the same physiological changes as after exposure to lack of oxygen (Fröhlich,[2] Boruttau[3]); the rate of conduction is slowed, the refractory period is prolonged, and repeated stimulation causes definite fatigue-effects; oxygen then restores the normal properties, but only in the absence of the anæsthetic. All of the phenomena of asphyxia appear during narcosis even in the presence of a good supply of oxygen, just as they do in an oxygen-free atmosphere in the absence of the narcotic (Fröhlich, Bondy,[4] Heaton).[5] Experiments by Ishikawa[6] on amœbæ gave analogous results; recovery from the inhibited or non-irritable condition, whether due to simple lack of oxygen or to the presence of a narcotic, requires the same condition, namely the presence of free oxygen. Other forms of inhibition, such as the heat-paralysis of the frog's central nervous system, are promoted both by lack of oxygen and presence of narcotics (Winterstein);[7] *i. e.*, anæsthesia acts in the same direction as lack of oxygen,—an indication that both conditions produce essentially the same physiological effect. Mansfeld[8] found that in the absence of oxygen tadpoles succumb more readily to anæsthesia than in its presence; the same is true of simple protoplasmic streaming in plant cells at temperatures of 30° and over (Zuckerkandl).[9] All of these facts seem to indicate that lack of oxygen produces essentially the same effects as anæsthesia; that the two actions are largely interchangeable, and hence capable of summation.

In general, however, it may be said, in criticism of such conclusions, that inhibition or prevention, under the influence of narcotics, of physiological processes which require oxygen, does not demonstrate that narcotics act directly and primarily upon oxidation-processes. The proper inference is rather that vital processes, *including* those which require free oxygen, are inhibited during anæsthesia. But anæsthesia may also inhibit physiological processes which are independent of free oxygen, as Winterstein has shown in his experiments on the narcosis of anaërobic animals like Ascaris.[1] Similarly the growth of yeast under anaërobic conditions is checked by anæsthetics in the same manner as in the presence of oxygen;[2] and the nerve cord of Limulus, which continues to send out impulses in the absence of free oxygen, is anæsthetized by ether in a typical manner.[3] Some more general condition which determines the rate of oxidations, as well as of other metabolic processes and cell-activities, is more probably the one directly affected in anæsthesia. This latter view would regard the suppression of oxidations as secondary rather than primary,—an effect rather than a cause of narcosis. During anæsthesia those processes which are directly dependent on oxidations are arrested, together with those not so dependent.

---

[1] Nagai, *Zeitschr. f. allg. Physiol.*, 1905, Vol. 5, p. 34.

[2] Fröhlich, *ibid.*, pp. 455, 468.

[3] Boruttau and Fröhlich, *Pflüger's Archiv*, 1904, Vol. 105, p. 444.

[4] Bondy, *Zeitschr. f. allg. Physiol.*, 1904, Vol. 3, p. 180.

[5] Heaton, *ibid.*, 1910, Vol. 10, p. 53.

[6] Ishikawa, *ibid.*, 1912, Vol. 13, p. 339.

[7] Winterstein, *ibid.*, 1905, Vol. 5, p. 342.

[8] Mansfeld, *Pflüger's Archiv*, 1909, Vol. 129, p. 69.

[9] H. Nothmann-Zuckerkandl, *Biochem. Zeitschr.*, 1912, Vol. 45, p. 412.

Various attempts have been made to refer narcosis to a decrease in the external supply of oxygen, or to an inability of oxygen to enter the cells. Thus anæsthesia as well as sleep were at one time popularly attributed to a condition of cerebral anæmia—an obviously untenable view, since neither condition is confined to animals with brain and circulation. That the anæsthetic hinders the entrance of oxygen into cells has recently been suggested by Mansfeld;[4] the solubility of oxygen in the lipoids of the plasma membrane—and hence its rate of entrance into cells—was held to be diminished by the solution of lipoid-solvents in the lipoids; and E. Hamburger[5] attempted to show that narcotic substances actually decreased the solubility of oxygen in lipoids. These views however must be regarded as unfounded (see Winterstein's criticism).[6]

[1] Winterstein, *Biochem. Zeitschr.*, 1913, Vol. 51, p. 165.

[2] *Cf.* Warburg and Wiesel, *loc. cit.*, p. 480.

[3] A. P. Mathews, *cf.* the article of Tashiro and Adams, *loc. cit.*, p. 451.

[4] *Loc. cit.*

[5] E. Hamburger, *Pflüger's Archiv*, 1912, Vol. 143, p. 186.

[6] Winterstein, *Biochem. Zeitschr.*, 1913, Vol. 51, pp. 158 *seq.*

The facts which offer best support to the oxidation theory of narcosis appear to be those which demonstrate an actual decrease in oxygen-consumption by isolated cells and tissues under the influence of narcotics. Thus Warburg and his associates have shown that oxygen-consumption by living cells of various kinds (red blood-corpuscles, sea-urchin eggs, bacteria, liver-cells, yeast-cells, the central nervous system) is decreased by various anæsthetics,[1] and the different narcotic compounds show the same order of relative action as in anæsthesia. For example, the several alcohols lower the oxygen-consumption of birds' erythrocytes by 50 per cent. in solutions of the following concentrations.[2]

TABLE VI.

Alcohol.	Concentration of Solution Depressing Oxidations 50 Per Cent.		Concentration for Anæsthesia of Tadpoles (Overton).
	Per Cent. (by Weight).	Molecular.	
Methyl	16	5*m*	0.52–0.62
Ethyl	7.3	1.6*m*	0.27–0.31
Propyl	5	0.8*m*	0.11
n-butyl	1.1	0.15*m*	0.038
i-butyl	1.1	0.15*m*	0.045
Amyl	0.4	0.045*m*	0.023

It is to be noted that these concentrations are much higher than those usually required for anæsthesia, as comparison with Overton's results (third column) will show. Vernon also found that anæsthetics decreased the oxidation of the indophenol reagent by fresh tissues. An especially interesting fact is that anæsthesia causes a similar though less marked decrease in oxidation by dead cells and by tissue-extracts, as has been demonstrated by both Warburg and Vernon.[3] This suggests that the anæsthetizing influence is exerted directly upon the oxygen-catalyzers of the cell; and recently a number of investigators have devoted special study to the inhibiting action of anæsthetics on oxidases.

---

[1] For a summary of these researches *cf.* Warburg, *München. med. Wochenschr.*, 1911, Vol. 58, p. 289; for the case of isolated tissues *cf.* Usui, *Pflüger's Archiv*, 1912, Vol. 147, p. 100; for microörganisms, Warburg and Wiesel, *loc. cit.*

[2] Warburg, *München. med. Wochenschr.*, *loc. cit.*; *Zeitschr. f. physiol. Chemie*, 1910, Vol. 69, p. 452.

[3] Warburg and Wiesel, *loc. cit.*; Vernon, *Journ. Physiol.*, 1912, Vol. 45, p. 197.

Vernon[1] has investigated the influence of various anæsthetics upon the activity of the indophenol oxidase of the vertebrate kidney. Enzymes which accelerate the oxidative formation of the blue dye, indophenol, from a mixture of α-naphthol and para-diamino-benzene are widely distributed in organisms; and Vernon's investigations on the distribution of this oxidase in the tissues of vertebrates indicate that a relation exists between the oxidase-content of a tissue and its general oxidative activity.[2] Various anæsthetics were found to decrease the oxidation and eventually to destroy the oxidase. The concentrations required to decrease activity by one half under otherwise constant conditions showed a close parallelism with those required to hæmolyze red corpuscles. In both cases the order of relative action was the same as for anæsthesia. The parallelism of lipoid-solubility with narcotic action appeared closer than that of surface-activity; and Vernon inclines to the belief that the narcotics exert their action chiefly by dissolving in the lipoids of the plasma-membrane and so altering the properties of this structure (possibly by interfering with the interaction of oxidase and peroxidase).[3] The oxidase-inhibiting concentrations are, however, far higher than the narcotizing, and correspond rather with the cytolytic concentrations, so that a direct connection seems doubtful. The work of Battelli and Stern[4] on the influence of anæsthetics on other tissue-oxidases (*e. g.*, a liver-oxidase which oxidizes succinic to malic acid) shows in general similar relations; in this case the inhibiting action was found to run closely parallel with surface-activity,—more so, according to Battelli and Stern, than with lipoid-solubility. It showed also a striking parallelism with a precipitating action on the nucleo-proteins of the tissue. As already stated, a similar precipitating action of anæsthetics has been investigated by Moore and Roaf, who agree with Battelli and Stern in regarding this action as an important factor in anæsthesia; the authors also attribute the action of anæsthetics not to their influence on lipoids alone, but rather to an alteration of the proteins of the tissue.[1] It seems clear that anæsthetics may directly inhibit oxidation-catalysis in tissues. But the objection again rises that the concentrations required for these effects greatly exceed those required for anæsthesia in living cells.

---

[1] *Loc. cit.*

[2] Vernon, *Journ. Physiol.*, 1911, Vol. 42, p. 402.

[3] *Loc. cit.*, 1912.

[4] Battelli and Stern, *loc. cit.*

The relation of oxidases to cell-respiration is still obscure. Present opinion inclines to the belief that these bodies are essentially peroxide-forming compounds (oxygenases) which are activated by other accessory substances present in cells. These bodies (activators or co-enzymes) may be other organic compounds (peroxidases); it appears also that in some cases inorganic salts, especially iron salts, may play this rôle (*cf.* Warburg).[2] There is some evidence that the combination of hæmoglobin with oxygen is of the nature of a peroxide; both hæmoglobin and hæmatin may cause bluing of guaiac and exhibit other oxidase-like properties.[3] Compounds which form unstable peroxide-like unions with oxygen are regarded by certain investigators as forming the essentially irritable part of the cell; the temporary stabilization of such compounds by any physical or chemical influence would thus be equivalent to anæsthetization. This view has recently been supported in this country by Mathews;[4] he regards anæsthesia as resulting from the formation of chemical unions between the anæsthetic and protoplasm; these unions are due to the residual valences of the anæsthetic (*i. e.*, the reserve powers of union left over in many compounds after the ordinary valences are satisfied, as seen in the formation of double salts, hydrates, etc.); the number of residual valences is variable, but tends to be higher in compounds with well-marked anæsthetic property. Mathews finds a general though not complete parallelism between the number of such valences in a compound and its narcotic power. He proposes the following explanation of anæsthesia: "The irritable substance in protoplasm is a molecular oxygen-protoplasmic union or a peroxide union, unstable and similar to oxy-hæmoglobin. By stimulation this unstable molecular union passes by molecular rearrangement into a stable form, oxidation taking place and carbon dioxide being directly or indirectly produced. The anæsthetic produces anæsthesia by occupying the oxygen-receptors of the cell, thus forming a non-irritable, dissociable, anæsthetic-protoplasm compound. The various facts of anæsthesia are explicable on this theory."

---

[1] *Cf.* Moore and Roaf, *loc. cit.* But Meyerhof (*Pflüger's Archiv*, 1914, Vol. 157, p. 273) finds that anæsthetics, in concentrations of the anæsthetizing or inhibiting order, have no influence on the osmotic pressure of protein solutions. This observation appears to be incompatible with the view that anæsthetics act by altering the aggregation-state of proteins.

[2] *Zeitschr. f. physiol. Chem.*, 1914, Vol. 92, p. 231.

[3] *Cf.* Moitessier, *Compt. Rend. Soc. Biol.*, 1904, Vol. 11, p. 373; Czylharz and Fürth, *Beitr. zur chem. Physiol. u. Path.*, 1907, Vol. 10, p. 358; McClendon, *Journ. Biol. Chem.*, 1915, Vol. 21, p. 275.

[4] A. P. Mathews, *Internat. Zeitschr. f. physik-chem. Biol.*, 1914, Vol. 1, p. 433.

Now it is a striking fact that the concentrations of the lipoid-solvent anæsthetic required to inhibit oxidations under the influence of enzymes, inorganic catalysts, or dead cells are far higher than those required for true anæsthesia, and are closely similar in their order of magnitude to the cytolytic concentrations. As already mentioned, Vernon found that the concentrations at which the activity of the kidney-oxidase began to be decidedly lowered corresponded closely with those found by Führer and Neubauer for hæmolysis; *i. e.*, in Vernon's words, "those which dissolve the lipoid membrane of the corpuscles." The concentrations required for anæsthesia in tadpoles are from eight to ten times lower. It thus seems doubtful that the anæsthetic effects can be referred to a direct inhibitory action on oxidases. The prompt and complete reversibility of anæsthesia is also a fact unfavorable to this view. According to Vernon's results, tissue-oxidases are rapidly destroyed by those concentrations of lipoid-solvent anæsthetics which reduce their activity to half its original value. We find, in fact, that in those cases where anæsthesia is associated with decrease of intracellular oxidations, the inhibitory effect is obtained in relatively low concentrations; while in order to induce an equal decrease of oxidations in tissue-extracts, or in disintegrated tissues or cells, the required concentrations must be much higher. In other words, destroying the *structure* of the tissue destroys its sensitiveness to the inhibiting action of low concentrations of the anæsthetic. This fact seems to indicate that the anæsthetic acts on living cells primarily by altering certain organized or structural elements, upon the condition of which the normal rate of oxidation and of other metabolic processes depends. Experiments on tissue-extracts indicate that the normal oxidations in living cells like muscle-cells are far more rapid and complete than can be effected through the simple agency of the oxidases present (Fletcher and Hopkins, Warburg, Battelli and Stern)[1]; the rôle of oxidases in cell-respiration may therefore well be a subsidiary one; and if so, the fact that anæsthetics arrest cell-activities in concentrations which are without direct influence on the oxidases may be understood. The essential change in anæsthesia would then be a reversible alteration of certain structural elements that control oxidations as well as other cell-processes.

Such a view would regard oxidases as accessory rather than primary factors in cell-oxidations. If this is true, there should be no direct parallelism between decrease of oxidations and anæsthesia; and it should be possible in certain cases to secure anæsthesia without influencing oxidations. There are in fact numerous instances of complete and typical anæsthesia unaccompanied by any essential decrease in the rate of oxidations. The anæsthesia of anaërobic animals and yeast-cells has already been cited; these instances, however, may be considered equivocal,—since oxidations are equally essential to metabolism in these organisms even though molecular oxygen may not take part in the reactions. But many cases are known where the activity of aërobic organisms or tissues may be profoundly inhibited by anæsthetics, while the rate of oxidation is unaltered or only slightly decreased. Such lack of parallelism was observed by Rhode and Ogawa for the influence of chloral hydrate on the isolated heart.[2] The case of cell-division in developing eggs is an especially clear one; here the rate of oxidation is relatively slight compared with that of active muscle-cells. Warburg found that phenyl urethane in concentrations of $m/2{,}000$ arrests cell-division completely in sea-urchin eggs (*Strongylocentrotus*), while leaving oxygen-consumption essentially unchanged; in order materially to decrease oxidations (by 40 per cent.) several times the minimal anæsthetic concentration was needed ($m/500$).[3] Similar observations were made by Loeb and Wasteneys[1] on the eggs of another sea-urchin (*Arbacia*). In order to arrest cleavage by cyanide (which directly inhibits oxidations) a concentration sufficient to lower the rate of oxidation to one third the normal was needed. The oxidations could be reduced to one half the normal without arresting cleavage. But in solutions of various anæsthetics (chloral, urethane, chloroform, methyl, ethyl, and propyl alcohols) of concentration sufficient to prevent cleavage entirely, the rate of oxidation was found to be only slightly decreased,—on the average by less than 10 per cent. In solutions of urethane, during the complete arrest of cleavage, the rate of oxidation was 98 per cent. of the control. When it is considered that oxidations may be decreased by much more than 10 per cent. (by means of cyanide, or by lowering the temperature a few degrees) without arresting cleavage, it seems clear that the slight decrease of oxidation observed in these experiments can stand in no causal relation to narcosis. It is an accessory and apparently an unessential effect. Very similar results were found in experiments with young fish embryos (*Fundulus*) at a stage when the musculature was well-developed, so that active contractions could be evoked by external stimulation (*e. g.*, by acidulated sea water). If unstimulated, the embryos lie quiet within the egg-envelope; the disturbing effects of variations in muscular activity are thus absent. It was found that complete chloroform-narcosis had little or no influence on the rate of oxidations; ether and butyl alcohol caused some decrease in oxidations (25 per cent. to 30 per cent. at the narcotizing concentrations); but in order to render the animals insensitive by direct inhibition of oxidation through cyanide, it was found necessary to reduce the oxidations to *one ninth* of their normal rate. In marine medusæ (*Gonionemus*) paralysis by cyanide required a decrease in oxidations from three to six times greater than that accompanying urethane narcosis.

---

[1] Fletcher and Hopkins, *Journ. Physiol.*, 1907, Vol. 35, p. 287; Warburg, *Zeitschr. f. physiol. Chem.*, 1911, Vol. 70, p. 413; *Pflüger's Archiv*, 1912, Vol. 145, p. 277; Battelli and Stern, *Biochem. Zeitschr.*, 1914, Vol. 67, p. 443.

[2] Rhode and Ogawa, *Archiv f. exper. Path. u. Pharm.*, 1912, Vol. 69, p. 200.

[3] Warburg, *Zeitschr. f. physiol. Chem.*, 1910, Vol. 66, p. 305; 1911, Vol. 70, p. 413.

The insensitivity of many cells and tissues to simple abstraction of oxygen or presence of cyanide is in striking contrast to their sensitivity to anæsthetics. Thus nerve-trunks only gradually lose irritability and conductivity in an oxygen-free atmosphere, or in cyanide solutions of considerable concentration (Dontas);[1] while the desensitizing effects of anæsthesia are rapid and complete. That the two effects are essentially different is further shown by the difference in the rate of recovery, which is much prompter in the case of anæsthesia. Apparently narcosis may decrease the oxidations in resting nerve trunks, as indicated by the output of carbon dioxide; this is seen in the experiments of Tashiro and Adams cited above, but the effect is comparatively slight and probably unconnected with the loss of irritability, since, as just seen, the nerve retains irritability in cyanide solutions for a long time. Compare also Winterstein's results, about to be described. Other similar instances are ciliary movement and protoplasmic rotation, both of which are only gradually checked by lack of oxygen or by cyanide, but instantly by narcotics. Recently Winterstein[2] has shown that the reflex irritability of the frog's isolated spinal cord may be entirely lost under anæsthesia without affecting the general oxidation-rate of the tissue; in fact during alcohol narcosis there was a slight but regular *increase* in oxygen-consumption. The narcotized cord differs from the non-narcotized in one chief respect; in the normal and incompletely narcotized cord stimulation causes increased oxygen-consumption; but during complete narcosis no such effect is seen; the oxygen-consumption during stimulation is the same as in the resting non-narcotized cord. Oxygen is, however, essential for the normal irritability of the cord; complete recovery from narcosis requires not only removal of the anæsthetic but also the presence of free oxygen. There may, however, be *partial* recovery even in the absence of oxygen. These facts illustrate how important oxygen is for the normal activity of the nerve-cell; but they also show that, given a supply of free oxygen, the consumption in the normal resting cord may be the same as in the narcotized cord. If narcosis were simply asphyxia, such a result would be inexplicable. A further fact inconsistent with the "asphyxia hypothesis" of narcosis is that after a narcosis lasting for days (9 days in one of

---

[1] Loeb and Wasteneys, *Journ. Biol. Chem.*, 1913, Vol. 14, p. 517; *Biochem. Zeitschr.*, 1913, Vol. 56, p. 295.

---

[1] Dontas, *Arch. f. exp. Path. u. Pharm.*, 1908, Vol. 59, p. 430.

[2] Winterstein, *Biochem. Zeitschr.*, 1914, Vol. 61, p. 81.

Winterstein's experiments with urethane) reflex irritability returns promptly on the removal of the anæsthetic. There is no evidence that nerve-cells can resist lack of oxygen for any such time. There is, however, ample evidence from other sides that during narcosis the normal resting oxidations of tissues continue uninterruptedly. Winterstein also finds that the oxidative removal of acid products of asphyxia takes place equally readily in normal and in narcotized nerve-cells. The central nervous system of the frog, which normally exhibits an alkaline reaction to litmus, becomes acid during asphyxia; if oxygen is then restored the alkaline reaction returns; but narcosis was found to have no influence on this effect; clearly therefore, narcosis does not interfere with these oxidations.[1]

Such observations should be correlated with those of Vernon, Warburg, Battelli and Stern cited above, indicating that tissue-oxidations are directly influenced only by relatively high concentrations of anæsthetics. Taken in conjunction with the other instances just cited, of anæsthesia with essentially unaltered rate of oxidation, they indicate that a direct suppression of intracellular oxidations (or asphyxia) is probably not the essential basis of anæsthesia. Apparently the latter condition does not depend on any alteration of purely chemical conditions, but on some influence exercised by the anæsthetizing agency on the structural or organized (or "living") substratum in which the chemical processes take place and by which their character and rate are controlled. The evidence of this will now be considered.

There appears to be a general relation between degree of organization and susceptibility to narcosis. Plants and lower organisms require higher concentrations of anæsthetic than higher animals (Overton); in vertebrates the cells most susceptible to narcosis are those of the higher brain-centers. Such cells are distinguished by high irritability and rapid variations in their activity, peculiarities which are undoubtedly a function of their special structure. It is true that if organization is destroyed many of the chemical processes of protoplasm (oxidations and fermentations) may still continue (autolysis), and may then be slowed by anæsthetics; but as already shown, much higher concentrations are required to produce such effects than to anæsthetize the intact living cells. Such facts indicate that when anæsthetics influence oxidative and other metabolic processes within the cell, they do so not directly, but through their influence upon *some specially sensitive intermediary*, which is a part of the organized structure of the cell and itself controls the rate of the intracellular chemical processes. It is this intermediary which is directly influenced by the anæsthetic. Its part may be compared to that of a sensitive starter or relay in a complex mechanical or electrical system.

---

[1] Winterstein, *ibid.*, 1915, Vol. 70, p. 130.

Various general considerations support this view. When an irritable element, *e. g.*, a muscle-cell, is stimulated by a mechanical impact, it is difficult to suppose that the primary effect of this impact is to hasten oxidative processes; it is true that an increase in oxidations does follow, but this effect represents a later stage in the complex sequence of interdependent processes constituting the response to stimulation (and of which the contraction is the most evident). If the muscle is previously treated for a short time with an anæsthetic, or if the magnesium or calcium-content of the medium is sufficiently increased, contraction no longer results. The entire sequence of processes normally following stimulation is prevented. It seems more probable that the *primary* event in the physiological sequence is the one directly interfered with; if this is prevented so also are the others. It further seems clear that in an irritable cell this primary or determinative change must be a *surface-change*; obviously that part of the irritable element which is directly in contact with the medium is the one first affected by the stimulus; and there is ample evidence that the direct action of many stimuli is confined to this surface-layer. Indirectly the activity of the whole cell is of course affected; but this must be by means of some influence transmitted from the surface throughout the cell-interior. The nature of this influence forms in fact the chief problem of the physiology of stimulation.

The fact that a surface-effect is sufficient to set in motion the whole complex apparatus of response in the cell-interior is a cardinal one in any theory of anæsthesia. Unmistakable evidence that this is the case is seen in the delicacy of the response which sensitive organisms like protozoa show to the contact stimuli of food particles or prey; also in the prompt response of many living cells to the presence of substances which are known from experiments on permeability not to penetrate the plasma-membranes. In general these membranes in their normal state are impermeable to the neutral salts of the alkali and alkali earth metals, as Overton showed; yet variations in the proportions of such salts in the tissue-media profoundly influence the activity and irritability of living cells. Increase in the magnesium or calcium salts of the medium may cause typical anæsthesia in muscle and nerve cells. Warburg and Harvey[1] have shown that cell-division in sea-urchin eggs may be arrested by weak solutions of sodium hydrate without the alkali penetrating the cell. A. J. Clark has made similar observations for heart muscle cells, and Harvey for the contractile cells of medusæ and for protoplasmic rotation in plant-cells.[2] Irritability and automatic activity may thus be abolished by substances to which the cell-surface is impermeable. The general facts of electrical stimulation also indicate that alteration in the electrical condition of the cell-surface is the primary event in stimulation. The investigations of Nernst and his successors in the theory of electrical stimulation show that the electrical current stimulates by changing the relative concentrations of ions on the opposite faces of the semi-permeable membranes enclosing the irritable elements,—*i. e.*, by altering the electrical polarization of the surface-film or the plasma-membrane. A characteristic electrical variation, the action-current—which is best explained as the result of alterations in the electromotor properties of the cell surface—also accompanies all forms of stimulation, and this electro-motor variation is prevented by anæsthetics. It may be held, therefore, with a high degree of probability that the primary event in stimulation is a *surface-process*, consisting in some physico-chemical alteration of the modified protoplasmic surface-film (plasma-membrane) which delimits irritable cells.[3]

---

[1] Warburg, *loc. cit.*; E. N. Harvey, *Journ. Exper. Zool.*, 1911, Vol. 10, p. 507.

[2] A. J. Clark, *Journ. Physiol.*, 1913, Vol. 46, p. xx; Vol. 47, p. 66; E. N. Harvey, *loc. cit.*, and Yearbook of Carnegie Institution, No. 10, 1911, p. 128.

[3] For a general discussion of the evidence bearing on this problem *cf.* my article "The Relation of Stimulation and Conduction in Irritable Tissues to Changes in

Lately many investigators have concurred in this view that alterations in the physico-chemical properties of the plasma-membrane form the essential basis of anæsthesia. Whatever condition alters this structure so as to make it less capable of undergoing the changes of permeability and of electrical polarization which normally accompany stimulation—and apparently other forms of cell-activity—has an inhibiting or paralyzing effect on the cell. This general view has been reached as the outcome of a large number and variety of investigations. Overton in 1904 pointed out that the paralyzing action of potassium salts on voluntary muscle must be referred to their action on the membrane, since plasmolytic experiments indicate that such salts do not penetrate into the cell interior.[1] The view that the physiological action of neutral salts is due primarily to their influence on the plasma-membrane was later strongly supported by Höber[2] on the basis of experiments on the influence of neutral salts on the demarcation-current potential of muscle. This may be influenced in the direction either of increase or decrease by treatment with isotonic solutions of sodium and other salts. Salt solutions, like those of potassium salts, which decrease this potential—*i. e.*, produce local negative variation—apparently do so by altering the colloids of the plasma-membrane and so increasing its permeability; in general such increased permeability to ions involves decrease in the electrical polarization of the membrane (*i. e.*, negative variation); increased polarization means an alteration of the membrane in the reverse direction, *i. e.*, of *decreased* permeability. These changes of polarization and permeability result from the altered condition of the colloids forming the membrane. The colloidal system of the membrane acquires in the presence of certain salts a less dense consistency ("Auflockerung") associated with increased permeability; and a denser consistency ("Verdichtung") in the presence of others, *e. g.*, sodium iodide, etc. The microscopic appearances observed

---

the Permeability of the Limiting Membranes," in *Amer. Journ. Physiol.*, 1911, Vol. 28, p. 197. Also, for a more elementary treatment, the articles "The Rôle of Membranes in Cell-Processes," and "The General Physico-chemical Conditions of Stimulation," in the *Popular Science Monthly*, 1913 and 1914.

[1] E. Overton, *Pflüger's Archiv*, 1904, Vol. 105, p. 176; *cf.* p. 207.

[2] R. Höber, *Pflüger's Archiv*, 1905, Vol. 106, p. 599.

in nerve-fibers treated with pure solutions of sodium and potassium salts support this conception.[1] Changes in the electrical condition of the cell are thus indices of changes in the colloids forming the plasma-membrane, and especially in the lipoids. Now, since the stimulation-process is always accompanied by electrical variations, it seems probable that this process is itself dependent on changes in the colloids of the plasma-membrane, involving changes of permeability; correspondingly, artificial alterations in the condition of these colloids should modify the irritability of the cell.[2] In an important paper published in 1907, entitled "Contributions to the Physical Chemistry of Stimulation and Narcosis,"[3] Höber applies this general conception to the problem of narcosis essentially as follows: Stimulation is associated with an alteration in the condition of the colloids of the plasma-membrane, involving a general increase of permeability; narcotics are those agents which prevent this alteration in the condition of the protoplasmic colloids and hence prevent stimulation. The colloids chiefly concerned are the lipoids; narcosis depends on the collection of lipoid-soluble substances in the lecithin of the plasma-membrane to a certain critical concentration, and on a prevention, by means of these substances, of the colloid-process normally concerned in stimulation. Höber showed that various anæsthetics (ethyl and phenyl urethane, chloral hydrate, chloroform, hypnon) do in fact check the action of rubidium and potassium salts in causing negative variation in frogs' muscle. They also prevent the effect of potassium sulphate in causing structural changes ("Auflockerung") in nerve. The anæsthetic thus *antagonizes* the salt-action,—just as it is known to antagonize the stimulating action. Similar effects may be produced by alkali earth salts, especially of calcium. According therefore to Höber's hypothesis, the physico-chemical basis of these antagonisms, and hence of anæsthetic action in general, is an alteration of the colloids, especially the lipoids, of the plasma-membrane, and a consequent change in the properties of this structure. The temporary increase of permeability, which according to Bernstein's theory of the bioelectric variations, forms an essential part of the stimulation-process, is thus rendered difficult or impossible.

---

[1] Höber, *Zentralblatt f. Physiol.*, 1905, Vol. 19, p. 390.

[2] For a fuller account of Höber's views see his general article on "the physico-chemical processes in stimulation" in *Zeitschr. f. allg. Physiol.*, 1910, Vol. 10, p. 173. Also his textbook, "Physikalische Chemie der Zelle und der Gewebe," 4th edition, 1914.

[3] *Pflüger's Archiv*, Vol. 120, p. 492.

Very clear and concrete indication that the stimulation of muscle is in fact associated with a temporary increase in the permeability of the plasma-membrane, and that anæsthetics act by preventing this increase, was afforded by my own experiments on the larvæ of the marine annelid *Arenicola*, carried out at Woods Hole in 1908.[1] This larva is a free-swimming trochophore one third of a millimeter long, possessing a well-developed musculature and swimming by cilia, and is peculiar in having its body-cells permeated by a brown water-soluble pigment. This pigment normally remains within the cells, but under conditions of increased permeability, as on death or under the influence of cytolytic substances, it diffuses readily into the sea-water and imparts a yellow tinge to the latter. It serves therefore as a convenient indicator of increase of permeability. If larvæ are brought suddenly from sea water into a pure isotonic solution of a sodium salt (*e. g.*, 0.6*m* NaCl), a strong muscular contraction at once results, accompanied by a well-marked loss of pigment; at the same time the cilia cease movement and soon afterwards undergo breakdown, and other toxic effects follow. If, instead of a *pure* solution of NaCl, a solution containing a little $CaCl^2$ or $MgCl^2$ is used, both changes are simultaneously prevented; neither contraction nor loss of pigment is shown; the cilia remain active, and normal swimming movements continue for a time; the general toxic action of the pure salt solution is also prevented. Thus the calcium prevents at the same time both the stimulating and the permeability-increasing action of the sodium salt. It also greatly diminishes the injurious action of the latter, *i. e.*, exerts anti-toxic action. Pure solutions of KCl also cause strong muscular contractions accompanied by loss of pigment; and the effect is similarly checked by the addition of $MgCl_2$. In mixtures of KCl and $MgCl_2$ both effects vary with the Mg-content of the solution in a closely parallel manner; solutions with relatively high Mg-content cause slight stimulation and slight loss of pigment, while in those of relatively high K-content both effects are well marked.

---

[1] *Cf. Amer. Journ. Physiol.*, 1909, Vol. 24, p. 14. *Cf.* also *ibid.*, 1911, Vol. 28, pp. 210 *seq.*

Entirely different effects from those of pure solutions of potassium and sodium salts are produced by pure solutions of magnesium salts. These exert typical anæsthetic action on *Arenicola* larvæ, as on other marine animals. When brought suddenly into pure isotonic $MgCl_2$ solution the larvæ show no contraction or loss of pigment; all muscular contraction immediately ceases, the body remains rigid and extended; the cilia are more resistant and remain active, and slow undirected swimming movements continue. On return to sea water muscular movement and other normal activities are at once restored. If larvæ are brought into isotonic $MgCl_2$ solution for a few minutes, and are then transferred into pure NaCl solution, the characteristic effects following transfer to the solution from sea water—stimulation and loss of pigment—are no longer seen; the organisms remain motionless and without apparent change. If they are then returned to sea water they show prompt revival. Apparently the treatment with $MgCl_2$ renders the plasma-membrane more resistant than normally to the permeability-increasing action of the pure NaCl solution, and at the same time the muscle-cells become refractory to stimulation. The following was the general conclusion drawn at the time from these facts: "$MgCl_2$ and similarly acting solutions appear to *decrease* the permeability of the tissues and so prevent the ionic transfer on which stimulation depends. The general action of anæsthetics consists in *decreasing the normal permeability*; stimulating agencies on the other hand have the reverse effect."[1] Later experiments with a variety of lipoid-solvent anæsthetics—alcohols, esters, ether, hydrocarbons—gave essentially similar results;[2] solutions of anæsthetics in pure NaCl solution, in every case where they prevented the stimulating action of the solution, also prevented the permeability-increasing action as indicated by loss of pigment; when they did not prevent this effect, they did not prevent stimulation. A general parallelism between prevention of permeability-increasing action and prevention of stimulation was thus shown. Anæsthetics were also found to prevent other effects depending on increase of permeability, such as the toxic effects of pure solutions of Na and K salts on sea urchin and starfish eggs, as well as on *Arenicola* larvæ,[1] and also the activation of unfertilized sea-urchin eggs by pure salt solutions (KCNS, NaI).[2] In general the anæsthetics appear to exert a *stabilizing* influence on the plasma-membrane, rendering it more resistant than normally to influences that tend to increase its permeability. To this stabilizing action both the inhibitory or anti-stimulating (anæsthetic) and the protective (anti-toxic) actions of both salts and lipoid-solvent anæsthetics are due.

---

[1] *Loc. cit.*, p. 44.

[2] *Amer. Journ. Physiol.*, 1912, Vol. 29, p. 372; 1913, Vol. 31, p. 255.

Recently a large body of evidence has accumulated from various sides indicating that anæsthetics either decrease the permeability normal to the resting cell, or render the plasma-membrane more resistant than normally to increase of permeability. Thus, according to Lepeschkin,[3] the entrance of dyes into plant cells (*Spirogyra*) is checked in the presence of low concentrations of ether and chloroform, and according to Szucs,[4] also by neutral salts. I have made similar observations on *Arenicola* larvæ.[5] These effects indicate a decrease in the general permeability of the plasma-membrane to diffusing substances; this change is apparently associated with a characteristic alteration in the density or physical consistency of the membrane, rendering it more than normally resistant to disintegrative or toxic agencies in general. Hence anæsthetics as well as neutral salts protect the cilia, pigment-cells and musculature of *Arenicola* larvæ against the injurious action of pure Na-salt solutions; the same is true of sea-urchin and starfish eggs. Similar observations are described by other authors. Arrhenius and Bubanovic[6] find that blood-corpuscles may be protected by anæsthetics against cytolysis in hypotonic solutions; and Traube has made similar observations for solutions of cytolytic substances (hæmoglobin).[1] The increase in permeability caused by pure solutions of Na-salts in fish eggs is also hindered by alcohol and ether, according to McClendon;[2] and Loeb has described similar effects of alcohol on *Fundulus* eggs.[3] The most conclusive evidence of a decrease in permeability during narcosis is however derived from experiments on the electrical conductivity of narcotized cells; this appears to be decreased during narcosis (McClendon, Osterhout, Höber and Joel). McClendon in 1910 found the conductivity of sea-urchin eggs to be decreased by chloroform, but did not investigate the phenomenon in detail. More extensive experiments have been carried out on plant cells by Osterhout,[4] who has succeeded in showing clearly that in the presence of moderate concentrations of anæsthetic the cells of the marine alga *Laminaria* undergo increase in electrical resistance, indicating decreased permeability to ions; on removing the anæsthetic the original conductivity returns. If too high concentrations of anæsthetic were used the result was quite different; conductivity underwent marked increase and the effects were irreversible; the tissue had in fact undergone injurious or cytolytic alteration. Evidently the change corresponding to anæsthesia is the reversible change of *decreased* conductivity, indicating decreased permeability. Similar observations on blood-corpuscles have recently been made by Joel under Höber's direction.[5]

---

[1] *Ibid.*, 1912, Vol. 30, p. 1.

[2] *Journal of Experimental Zoology*, 1914, Vol. 16, p. 591. *Cf.* also *Journ. Biol. Chem.*, 1914, Vol. 17, p. 121.

[3] Lepeschkin, *Ber. d. deutsch. Bot. Gesellsch.*, 1911, Vol. 29, p. 349.

[4] Szucs, *Jahrbuch f. wissenschaftliche Botanik*, 1912, Vol. 52, p. 85.

[5] *Amer. Journ. Physiol.*, 1909, Vol. 24, p. 26.

[6] Publications of Nobel Institute, 1913, No. 32 (quoted from Höber's "Physik. Chem. d. Zelle," p. 466).

Taken in its entirety the foregoing evidence indicates that under the influence of a narcotizing agent the plasma-membrane undergoes an increase in its general stability or resistance to alteration; stimulation is prevented because this effect requires ready and rapid variations of permeability, and of these the stabilized membrane is no longer capable. Associated with this general stabilization is a decrease in the permeability to diffusing substances; the cell is more completely shut off from the disturbing effects of environmental changes. There is a possibility that the permeability to gases like carbon dioxide and oxygen is also decreased, but this remains uncertain at present.

---

[1] Traube, *Biochem. Zeitschr.*, 1908, Vol. 10, p. 371. See also the experiments of G. H. A. Clowes, *Proc. Soc. Exper. Biol. and Med.*, 1913, Vol. 11, p. 8.

[2] McClendon, *Amer. Journ. Physiol.*, 1915, Vol. 38, p. 173.

[3] J. Loeb, *Biochem. Zeitschr.*, 1912, Vol. 47, p. 127; *cf.* p. 155.

[4] *Cf.* Osterhout, *Science*, N. S., 1913, Vol. 37, p. 111; also "Quantitative Researches on Permeability" in "The Plant World," 1913, Vol. 16, p. 129.

[5] Joel, *Pflüger's Archiv*, 1915, Vol. 161, p. 5.

It is important to note that changes in the resistance of plasma-membranes, probably essentially similar to those underlying anæsthesia, may occur under completely normal conditions, as I have recently found in experiments on dividing sea-urchin eggs. It had been shown earlier by Lyon,[1] Spaulding,[2] and Mathews[3] that during the normal cycle of cell-division these cells are much more susceptible to poisons (cyanide, acids, ether, and K-salts) at the time when the cleavage-furrow is forming, than in the period preceding or following cleavage; during cleavage there is also an increased output of CO_2.[4] A rhythm of susceptibility to poisons and of CO_2-production is thus associated with the rhythm of cleavage. This condition suggested the possibility that the essential underlying condition of this rhythm might be a rhythmical change in the properties of the plasma-membrane; and in a series of experiments with dilute sea water it was found that the eggs do in fact undergo cytolysis in hypotonic media far more readily at the time when the furrow is forming than during the intervals between cleavage.[5] In other words, the membrane is relatively sensitive to osmotic disruption during cleavage, and relatively resistant during the intervals between cleavage, *i. e.*, at the resting times when the cell is relatively highly resistant to the action of poisons. This normal state of relatively high stability may be compared to the condition which is imparted to the membranes of irritable cells by anæsthetics. Increased resistance to hypotonic solutions in the presence of anæsthetics has been observed by Arrhenius and Bubanovic in blood corpuscles, as already cited.

How does the anæsthetic produce this alteration in the properties of the membrane? Attempts to find parallels between the effects of anæsthetics on living cells and on colloidal suspensions of lipoids have not given entirely consistent results. Höber and Gordon[1] found that lecithin suspensions containing ether, chloroform, chloral, or amyl alcohol were less readily precipitated by calcium and barium salts than the control suspensions, *i. e.*, were protected against precipitation or *stabilized* by the anæsthetic; and they refer to this phenomenon as "narcotization of the plasma-membrane colloid lecithin." Koch and McLean,[2] however, find that no such effect is general with anæsthetics; they find chloral and ether indifferent, while chloroform protects lecithin against precipitation by $CaCl_2$; on the other hand, alcohol and paraldehyde further precipitation. These discrepancies are probably due to differences of concentration. Recent experiments of my own with a large number of anæsthetics have shown that with the great majority of such compounds lecithin emulsions may be protected to a greater or less degree against the precipitating action of $CaCl_2$ and HCl; *i. e.*, a concentration of electrolyte just sufficient to cause precipitation in the absence of the anæsthetic, fails to do so or does so incompletely in its presence. The concentrations required to produce this stabilization are however much higher than the anæsthetizing concentrations, and the different compounds vary greatly in effectiveness. The usual increase of action with increasing molecular weight in members of homologous series (alcohol, esters) was seen. But with certain compounds little or no protection was found, while a few (ethyl and methyl alcohols, and in part isopropyl alcohol, acetonitrile, and in part paraldehyde) definitely furthered precipitation. The compounds which distinctly prevented precipitation included higher alcohols (*n*-propyl, *n*-butyl, *i*-amyl, capryl), esters (ethyl nitrate, propionate, butyrate; methyl, ethyl, and phenyl urethanes), hydrocarbons (chloroform, carbon tetrachloride, nitromethane, benzol, toluol, xylol); ethyl ether, chloral hydrate, chloretone, chloralose, acetanilide, phenyl urea. In general, therefore, lipoid-solvent anæsthetics exhibit a stabilizing influence against precipitation by electrolytes, but this influence is not always present. It may depend upon altered electrical polarization of the colloidal particles, or possibly upon increase of viscosity; but it is doubtful if in itself it forms a factor of any importance in true anæsthesia. The effective concentrations are too high and the effect is too variable.

---

[1] E. P. Lyon, *Amer. Journ. Physiol.*, 1902, Vol. 7, p. 56.

[2] E. G. Spaulding, Biol. Bull., 1904, Vol. 6, p. 224.

[3] A. P. Mathews, Biol. Bull., 1906, Vol. 11, p. 137.

[4] Lyon, *Amer. Journ. Physiol.*, 1904, Vol. 11, p. 52.

[5] R. S. Lillie: Amer. Journ. Physiol., 1916, Vol. 40, p. 130.

---

[1] Höber and Gordon, *Beitr. zur chem. Physiol. u. Pathol.*, 1904, Vol. 5, p. 432.

[2] Koch and McLean, *Journ. of Pharm. and Exper. Therapeutics*, 1910, Vol. 2, p. 249.

A probably more significant physical change caused by lipoid-solvent anæsthetics is an increase in the viscosity of lecithin suspensions. Handowsky and Wagner[1] observed such an increase of viscosity in the presence of alcohol; and A. Thomas, working in my laboratory, has confirmed and extended these observations.[2] Thomas observed that in the case of ether the increase of viscosity in concentrated emulsions of lecithin might go so far as to cause true gelation. I have found that this effect is very general; it is well shown in lecithin emulsions of 10 to 12 per cent. concentration; these are highly viscous, but still fluid in consistency; on the addition of many anæsthetics this consistency changes to that of a soft more or less coherent and elastic gel, in some cases of sufficient firmness to permit the inversion of the test-tube without spilling. Well-marked gelation was observed with the following compounds: alcohols (*n*-propyl, *i*-propyl, *n*-butyl, *i*-amyl, capryl), esters (ethyl formate, acetate, propionate, butyrate, nitrate), ethyl ether, ethyl chloride, ethyl bromide, chloroform, carbon tetrachloride, benzol, toluol, xylol, chloretone, chloral hydrate, chloralose, paraldehyde. On the other hand, certain very efficient narcotics had no such effect, *e. g.*, methyl, ethyl and phenyl urethanes, ethyl alcohol, nitromethane and acetonitrile. Gelation, however, is to be regarded as simply an end-effect of increase in viscosity; the latter change is the essential, and this is perhaps the most general and significant of the purely physical changes produced by lipoid-solvent anæsthetics in colloidal suspensions of lipoids. Such a change will have in general a retarding influence on physical and chemical processes taking place in such a system; it will decrease diffusion-rates and hence reaction-velocities depending on such rates; more energy, mechanical or other, is required to produce any kind of change in a highly viscous system. A general hindrance to diffusion would express itself in a decrease of permeability and of electrical conductivity. The influence of changing viscosity on the electrical conductivity of lipoid suspensions remains to be studied. Recently Loewe[1] has investigated the influence of a number of anæsthetics on the electrical resistance of solid artificial membranes impregnated with lipoids, and he has found that in some cases they cause decided decrease of conductivity, —a change to which he refers as "narcosis of the membrane." These results recall those of Osterhout on living plasma-membranes, but whether the anæsthetic effect in living cells is so direct as Loewe's experiments would indicate seems doubtful. Clowes's recent interesting conception of an interchangeability in the relative positions of the lipoid and aqueous phases of the protoplasmic emulsion—so that either the one or the other, according to conditions, may form the external or continuous phase —may throw a much-needed light on these phenomena. He suggests "that anæsthetics function by promoting the continuity of an external fatty or lipoid phase. The solubility of this lipoid film in adjacent aqueous phases being lowered, its permeability to water-soluble substances would be diminished. Since certain vital processes presumably depend upon intermittent intercommunication between adjacent aqueous phases, it may well be imagined that a temporary interruption in this communication would result in anæsthesia" (*loc. cit.*, p. 10). Such a change would involve both decreased permeability to water-soluble substances and greater stability of the membrane.

---

[1] Handowsky and Wagner, *Biochem. Zeitschr.*, 1911, Vol. 31, p. 32.

[2] A. Thomas, *Journ. Biol. Chem.*, 1915, Vol. 23, p. 359.

On the whole it appears highly probable that lipoid-solvent anæsthetics cause their effects through some purely *physical* change in the cell-system—more particularly in the plasma-membrane. Their chemical inactivity as a class indicates this. Processes like solution and adsorption, rather than chemical combination, probably determine their action in most cases. It is, however, best not to be too dogmatic on this point, since the distinctions between solution, chemical combination, and adsorption are probably not absolute. There is always the possibility that in certain cases the anæsthetic may form some chemical combination which interferes with chemical or other changes necessary to stimulation; the inactivation of hæmoglobin by carbon monoxide may serve as an illustration of how this is possible. Höber[2] has recently suggested that in true reversible narcosis the direct effect of the anæsthetic is limited to a surface-action or adsorption affecting all of the colloids of the membrane; in higher than the narcotizing concentrations the solvent action of the anæsthetic upon the lipoids of the membrane assumes importance and leads to disruptive and hence irreversible effects. This view attributes the destructive action of anæsthetics in high concentrations to a process different from that underlying true anæsthesia. Meyer's experiments on the effects of temperature, already cited, indicate, however, that solution of the anæsthetic in the lipoids is an essential factor in the total physiological effect. It is conceivable that a slight solution of this kind, combined with the general increase in the viscosity of the protoplasmic surface-layer, may increase the stability of the membrane; while a more pronounced solution may lead to direct solvent or other structure-altering effects which destroy its semipermeability and so cause cytolysis.

---

[1] S. Loewe, *Biochem. Zeitschr.*, 1913, Vol. 57, p. 161.

[2] Höber, *Deutsche medizinische Wochenschrift*, 1915, No. 10.

It must not be forgotten, however, that lipoid-solvents form only one class of anæsthetics. Apparently all substances or conditions which stabilize the membrane in a reversible manner may exert anæsthetic action. Anæsthesia due to neutral salts, cold, or the electric current, may be understood from this point of view; salts or low temperature may stabilize the membrane by causing gelation or other direct changes of aggregation in the colloids; the electric current by altering its state of electrical polarization. Some years ago I expressed this general view as follows: "Anæsthetic action is due primarily to a modification of the plasma-membrane of the cells or irritable elements, of such a kind as to render these membranes more resistant towards agencies which under the usual conditions rapidly increase their permeability; cytolysis and stimulation, both of which depend on such increase of permeability, are hence checked or prevented. Decrease in the readiness with which the permeability is increased thus involves for an irritable tissue decreased irritability; this effect is produced by various salts, *e. g.*, of magnesium, and by ether and other lipoid-solvent anæsthetics in certain, not too high, concentrations. . . . It seems clear that for irritable tissues the state of the lipoids in the plasma-membrane largely determines the readiness with which changes of permeability—and of the dependent electrical polarization—are induced by external agencies. Slight permeation of the lipoids with a lipoid-solvent apparently often facilitates such changes, and hence increases irritability; the present of more lipoid-solvent renders a change of permeability difficult, hence the protective or anæsthetic action; while concentrated solutions of lipoid-solvents disrupt the membrane and produce cytolytic or irreversible alterations in the cells; hence such substances in higher concentrations are markedly toxic."[1]

The question of just how this stabilizing influence is exerted is the critical one. An irritable element like a nerve-fiber or muscle-cell responds to a slight local electrical stimulation or mechanical impact; this response is apparently associated with a rapid and reversible increase of membrane-permeability; to this latter change the electrical variation is apparently due. It is this membrane-change, with the associated variation of electrical polarization, which appears to be the primary physiological event in stimulation; it spreads rapidly over the whole membrane, and the other consequences of stimulation (contraction, increased oxidation, etc.) follow upon this surface-change. The question thus involves the whole problem of the physiology of stimulation. This is not the place for a detailed discussion of the various questions implicated in this central problem. It is evident, however, that the whole process of stimulation depends on the local initiation of the excitation-state, and on the rapid conduction of this state from the point of stimulation so as to affect the entire element.[2] All of these processes depend on the physical and chemical condition of the membrane; hence altering this condition alters the whole stimulation-process.

According to this conception the sensitivity of the membrane to changes of electrical polarization is its most characteristic peculiarity.[3] The basis of this sensitivity remains to be determined. It would appear that the peculiar properties of the membrane depend upon its being a *living* structure, the seat of a specific metabolism. That the characteristic semi-permeability depends on this latter peculiarity is seen in the fact that the death-process, however induced, is always associated with a marked increase in the general permeability and electrical conductivity of cells. In other words, the normal semi-permeable condition—involving, as it must, a certain constancy in the composition and physical state of the surface-film—is maintained only so long as the cell remains alive. This fact shows that semi-permeability, and the electrical polarization which is associated with this, are not simply static properties of the plasma-membrane, but are functions of a specific metabolic activity—including probably oxidations in most cases—which maintains constant the physico-chemical characteristics of the surface-layer of protoplasm. In the irritable element this metabolism appears to be altered in a definite manner by changes in the electrical polarization of the membrane; and along with these chemical alterations go alterations of permeability and, secondarily, of electrical polarization. These latter involve the production of local electrical circuits which traverse and hence stimulate the adjoining inactive portions of the irritable element; in this manner the state of excitation *spreads*, and the whole element is stimulated.[1] But this is the case provided only that the membrane retains its normal sensitivity to changes of electrical polarization; if it has previously been rendered resistant by an anæsthetic, no such effect follows; the element as a whole then shows itself irresponsive to stimulation.

---

[1] BIOLOGICAL BULLETIN, 1911, Vol. 22, p. 328; *cf.* p. 331.

[2] For a more special discussion of the conditions of *conduction*, as distinguished from the other features of the stimulation process, *cf.* my two recent papers in the *Amer. Journ. Physiol.*, 1914, Vol. 34, p. 414, and 1915, Vol. 37, p. 348.

[3] *Cf.* the concluding section of my paper on antagonisms between salts and anæsthetics, *Amer. Journ. Physiol.*, 1912, Vol. 29, p. 391 *seq.* "In anæsthesia it is to be assumed that the membrane is so altered that it fails to respond to a change in its electrical polarization by an increase in its permeability" (p. 393).

---

[1] For a fuller discussion *cf.* my papers on the conditions of conduction in irritable tissues, already cited.

THE NATURE OF THE POLYHEDRAL BODIES FOUND IN INSECTS.[1]

R. W. GLASER AND J. W. CHAPMAN.

CONTENTS.

1. Introduction 367
2. The polyhedral bodies 369
3. Origin of the polyhedral bodies 373
4. Bio-chemical observations 376
5. Summary 382
6. Bibliography 383
7. Explanation of Plates 386, 388

INTRODUCTION.

A very large group of lepidopterous larvæ is subject to a class of infectious diseases known as the "polyhedral diseases." Whether the organism concerned in their production is identical or not for all the species of insects affected must remain a matter of conjecture till further work allows us to venture an interpretation. One thing, however, is certain, namely that curious crystal-like structures called "polyhedral bodies" or "polyhedra" are always associated with the type of diseases we are here discussing. Although these polyhedra may vary considerably in size and somewhat in shape in the different species of insects, nevertheless, they are always specific for a certain type of malady.

Wahl, followed by Prowazek and Escherich, consider the polyhedral diseases as distinct and absolutely divorce them from the fungous, protozoan and bacterial affections of insects. We believe that the erection of a separate group to embrace all of the polyhedral diseases is an excellent plan and receives our sympathetic endorsement, for the reason that the confusion of all of the insect diseases is still common amongst entomologists. We venture to say that there are scarcely two entomologists in America who know the difference or similarity between any of the diseases expressed by such terms as Muscardine, Pébrine, flacherie, lethargia, maladie de morts-blancs, schlaffsucht, faulsucht, fettsucht, polyhedral disease, wilt, wipfelkrankheit, jaundice and gelbsucht.

---

[1] Contribution from the entomological laboratory of the Bussey Institution in coöperation with the U. S. Bureau of Entomology (Bussey Institution No. 115).

We do not wish to dwell upon the differences or similarities between all of these maladies, but will confine ourselves solely to the group of polyhedral diseases under which are included the four commonest manifestations, viz., wilt, wipfelkrankheit, jaundice and gelbsucht. Wilt is the vernacular term used in America for the polyhedral diseases. It is a name suggestive of post-mortem aspects, but unfortunately a number of caterpillar diseases distinct from wilt on superficial examination also have a similar post-mortem appearance. Moreover, a number of diseases common to plant pathology are labelled with the same term. We do not think it wise to eliminate the name, however, for the reason that it has been used so long and "wilt" certainly means more to field entomologists and foresters than "polyhedral disease" which is only significant to laboratory workers. The term "Wipfelkrankheit," which is used for a similar affection of nun moth caterpillars in Germany, is a very suggestive name for the reason that when the animals are in the last stages of the disease, they congregate in masses at the tops of the trees or "wipfeln" and die hanging by their prolegs. The name "Wipfelkrankheit" has the further advantage in that it is not applied to any other plant or animal disease. "Gelbsucht" or "jaundice" are terms used to designate a polyhedral disease in silkworms. The two terms are descriptive of the clinical picture of the diseased worms and are not used for any other affection known to pathology.

The larval stages of the following species of lepidoptera have been examined by us and found to be susceptible to the polyhedral diseases. Animals which can only be infected experimentally have been omitted, *i. e.*, the list comprises only those which besides being capable of experimental infection also have the disease or diseases in a state of nature.

I. Saturnidæ.
  1. *Hemileuca maia* Drur.
II. Arctiidæ.
  2. *Apantesis virgo* Linn.

III. Noctuidæ.
  3. *Leucania unipuncta* Halw.
  4. *Noctua clandestina* Harris.
  5. *Autographa brassicæ* Riley.
IV. Lymantridæ.
  6. *Porthetria dispar* L.
  7. *Lymantria monacha* L.
  8. *Orgyia leucostigma* S. and A.
V. Lasiocampidæ.
  9. *Malacosoma americanum* Fabr.
  10. *Malacosoma disstria* Hubn.
VI. Bombycidæ.
  11. *Bombyx mori* L.
VII. Dioptidæ.
  12. *Phryganidia californica* Packard.
VIII. Pieridæ.
  13. *Colias philodice* Godart.

From a perusal of the above list it will be seen that the polyhedral diseases are very widely distributed and affect some of our most important economic insects. We have estimated that epidemics of polyhedral diseases at certain times kill off from 30 to 70 per cent. of some of our most noxious pests. This is especially true, as we have found in connection with our studies on the gipsy moth, tent caterpillars and army worms. The polyhedral diseases contribute much more to the control of certain of our noxious caterpillars than the combined efforts of all their hymenopterous and dipterous parasites. Therefore, we believe that these diseases merit a serious consideration from all points of view.

The Polyhedral Bodies.

Caterpillars dead from wilt are usually found on some elevated place hanging by their prolegs. Dead nun moth caterpillars are found hanging from the very highest branches of a conifer, dead gipsy moth caterpillars are found hanging anywhere on the trunk of a tree or on a branch; the favorite dying places of the American tent caterpillars being usually in close proximity to the nest on the branches of an apple or cherry tree. The army worm seeks the tip of a grass blade and succumbs thereon. An innate desire to reach an elevated place on their favorite food plant always seizes the diseased insects prior to death. We have never observed animals in the last stages of wilt descend their food plants. So far we are unable to offer any explanation which would satisfactorily assist in analyzing this ascending instinct. A short time after death, the animals become deliquescent. At the slightest touch the skin ruptures and a dark brown liquid oozes out. In some species such as the American and forest tent caterpillars this liquid is pink shortly after death and becomes dark brown later. The corpses will be practically odorless if they have hung but a short time and before septic bacteria have gained a foothold.

If some of the brown liquid from a dead caterpillar is examined microscopically with a high-power dry or oil-immersion lens, it will be found to contain, besides the elements of disorganized tissues, myriads of polyhedral bodies of various sizes. (Fig. 1, Plate 1.) Certain polyhedra have been found to measure ½ μ and less in diameter while still others reach the size of 15 μ. The average polyhedron of the gipsy moth caterpillar measures 3.4 μ in diameter. The bodies in this species are larger than those in any other form we have hitherto examined. The average size of nun moth caterpillar polyhedra measure 2.65 μ in diameter; those of the forest tent caterpillar 2.6 μ and so on until we come to *Phyrganidia californica* and the tussock moth caterpillars in which the average diameter has been found to be 1.6 μ and 1.5 μ respectively. Thus it is seen that the average polyhedron varies greatly in size in the different species. As stated previously the sizes of the polyhedra within one species or even within one animal (gipsy moth: ½ μ–15 μ) varies also. There exists a striking similarity between the shapes of these bodies in the different species but some variation within a particular species or even within the same animal can be observed. In general the form is that of a polyhedron with more or less rounded angles. They never assume the shape of a perfect sphere, and an actual geometric outline has never been observed except in the silk worms where almost perfect octahedra are found. The polyhedra are highly refractive, and on focusing are seen to have a denser center differentiated from a somewhat lighter periphery. Sometimes within the bodies concentric layers like those of an onion are observable. Often two polyhedra are seen adhering to one another as if in the act of dividing, but an actual division in a hanging-drop has never been observed. When pressure is applied to the cover glass, the polyhedra crack very readily into a number of pieces, and often without the application of pressure the same fragmentation may be observed to occur somewhat more slowly. In the latter case a notch appears at one side of the polyhedron which gradually lengthens into a line progressing slowly toward the other side, much like the cracking of ice. Usually before the line has completely separated the two halves other lines appear, and soon the entire polyhedron is divided into a number of pieces, which may separate or may stick together in a rosette-like fashion. At no time was anything observed to come out of the polyhedra when they cracked in this manner. If the cover glass is moved while applying a little pressure, one half of the polyhedron may sometimes be folded upon the other half without the cracks appearing, showing that it is composed of a tough substance and is not at all brittle like inorganic crystals.

The only objects in a fresh preparation with which one could possibly confuse the polyhedra are the fat globules and urate crystals, but with a little practice these may be readily distinguished. Fat globules are perfectly spherical and are therefore unlike the polyhedral shape of the bodies in question; but when in doubt, Sudan III was used, for in this stain the fat globules become red, while the polyhedra remain colorless. The urate crystals are often more acutely angular or are of an entirely different shape from the polyhedra and are frequently traversed by radiating lines.

Besides polyhedral bodies, fat globules, and urates, a smear from a newly "wilted" caterpillar contains cellular débris, hairs and pigment granules. The pigment granules must not be confused with bacteria, for many of them superficially resemble these organisms very closely. When a preparation is dried, mounted, and examined under oil, the pigment granules of the gipsy moth may easily be confused with small micrococci, owing to the fact that they are usually arranged in pairs. As a matter of fact, a smear made from a recently wilted caterpillar is almost devoid of bacteria, and in many cases none at all can be found. If bacteria are present, they have escaped into the body cavity through rupture of the intestine and bear no direct etiological relation to the disease.

In fixed and stained smears a number of things can be demonstrated to advantage within the polyhedra. Fixation was accomplished either by passing the preparation through a flame or by placing it in absolute alcohol for a few minutes. The smears were then stained in Giemsa's solution for 12 hours or were stained for a shorter period with one of the following dyes: Methylene blue, trypan blue, gentian violet, carbol fuchsin, Bismarck brown, or iron hæmatoxylin. When iron hæmatoxylin was used, the preparation was first mordanted in a 4 per cent. ferric-alum solution for two or three hours. After staining, the preparations were sometimes quickly passed through the alcohols to xylol before mounting. This not only clears everything, but dissolves away all the fat on the slide and thus increases the transparency of the preparation. Gipsy moth polyhedra are rather resistant to stains in general and usually color along the periphery only, unless the stain is applied for a long time. When this is done one may succeed in staining the entire polyhedron, especially after the use of some mordant like ferric alum before hematoxylin or anilin water before gentian violet. Steaming the preparation with a stain like carbol fuchsin has also given good results. When properly stained, one of three conditions is observed: First, the polyhedral bodies are uniformly stained so that nothing can be detected within them; or second, a uniformly darker staining central mass can easily be differentiated from an almost unstained outer substance; or, third, many little refractive, reddish granules are seen within the polyhedra. An actual differentiation between what might be interpreted as nuclear and cytoplasmic material within the polyhedra never occurs. Therefore, in accounting for the staining reactions we believe that at times the polyhedra have a central granular or homogeneous substance easily distinguishable from an outer tougher substance which is more resistant to the dyes. This varies a great deal, however, and sometimes the periphery takes the stain more readily than the underlying strata. From these staining reactions it becomes apparent that the polyhedra are complicated in structure, and do not therefore differ essentially from what Bolle and Prowazek found to be true of the silkworm polyhedra. Our observations on the staining reactions also show that morphological studies do not enable us to regard the polyhedral bodies as organisms. We believe that the polyhedra are protein degeneration-products of the disease. The staining reactions have demonstrated that they are not simple crystals, but complicated in structure, and have a tough outer layer. Consequently, and for a number of other reasons, we do not believe them to be true crystals and therefore choose to call them pseudo-crystals. The variations in their staining reactions which one obtains at times can well be accounted for by assuming that one is dealing with different stages in the synthetic process of pseudo-crystals. Another matter militating against the idea that the polyhedra are organisms is the fact that Glaser ('15) and Chapman and Glaser ('16) have experimentally demonstrated the possibility of infecting healthy gipsy moth caterpillars with wilt material from which the polyhedral bodies were removed by passing the virus through Berkefeld Grade "N" candles. We have shown that wilt is caused by a filterable virus and believe that the polyhedra arise as a reaction against the invasion of this virus.

ORIGIN OF THE POLYHEDRAL BODIES.

Studies on sectioned gipsy moth, army worm and tent caterpillar material have shown that the polyhedra originate within the nuclei of the hypodermal, fat, tracheal matrix, and blood cells. (Fig. 2.) In the true army worm, however, polyhedra are at times formed within the nuclei of intestinal epithelial cells. We have been utterly unable to find the bodies within the nuclei of muscle tissue, Malpighian tubes, ganglia, nerves, œnocytes, salivary glands, gonads and within the intestinal epithelial cells of all forms except the true army worm.

The formation of the polyhedral bodies within the nuclei of the four tissues above mentioned and the visible changes taking place within these nuclei may be described as follows: The first indication of a diseased nucleus seems to consist in the flowing together of the chromatin into a lump in the middle. Then out of the achromatic substance the polyhedra arise as very minute individuals. (Fig. 2, Plate I.) They gradually increase in size and probably most of the chromatin is also used up during the synthetic process. As the polyhedra grow, they become more and more refractive, stain with difficulty, and the nucleus becomes hypertrophied. The late stages of these hypertrophied nuclei are more than twice as large as the largest normal nucleus. (Fig. 2, Plate I, and 3, 4 and 5, Plate II.) This swelling of the nucleus is due to the increase in size of the polyhedral bodies which stretch the nuclear membrane. During the earlier stages of the disease the polyhedra are somewhat rounder than the larger ones found later on prior to death. This can be accounted for by the fact that, as the polyhedra grow, they become so closely packed within a nucleus that they press upon one another and thus the more or less polygonal shape is produced. As the polyhedra grow and become more refractive the remains of the chromatin lump disappears and there remains simply the nuclear membrane enclosing the polyhedra. (Fig. 2, Plate I, and 3, Plate II.) Finally the nucleus disintegrates, and the polyhedra are found free in great numbers in smears of dead caterpillars. (Fig. 6, Plate II.) We believe (and this belief is based on morphological, chemical and experimental evidence) that the polyhedral bodies are degeneration-products of the disease—products of nuclear disintegration. This view may not seem so improbable if one reviews some of the literature dealing with a few of the diseases in higher animals.

Hydrophobia (Rabies).

In 1903, Negri described certain bodies occurring in the nervous system of animals dying of rabies. The bodies seem to be specific to the disease, and are of great assistance for diagnostic purposes. The Negri bodies vary in size, measuring from .5 to 25 μ. They are round, oval or angular in outline and are found in the protoplasm of the nerve cells and their processes. The bodies occur in all parts of the nervous system, but are most common in the Purkinje cells of the cerebellum and especially in the cells of the cornu Ammonis. The virus of hydrophobia passes through the coarser Berkefeld and Chamberland filters, and these filters exclude the Negri bodies. The most generally accepted view at present is that the Negri body is a cellular reaction against the invasion of the filterable virus. The virus of hydrophobia has not been cultivated.

Variola and Vaccinia (Smallpox and Cowpox).

In 1892 Guarnieri described certain cellular inclusions in variola and vaccinia which are specific for these two diseases. The bodies are from 1–8 μ in diameter, and round, oval, or sickle-shaped. They lie in the cellular spaces, often in close proximity to the nucleus, and can be demonstrated in vaccine pustules as well as in the experimental lesions produced in the rabbit's cornea. The virus of variola and vaccinia passes through the coarser porcelain (Chamberland) filters. Most authorities regard the Guarnieri bodies as the effect of a specific reaction of epithelial cells against the virus. The virus has not been cultivated.

Trachoma.

In trachoma certain granules are found in the cytoplasm of the inflamed epithelial cells which cover the conjunctiva. Bodies similar to the trachoma bodies have also been found in other inflammations of the conjunctiva, and are therefore thought not to be specific of trachoma. The trachoma bodies are regarded as the product of mucous secretion under pathological conditions. The virus passes through Berkefeld filters. It has not been cultivated.

Cellular inclusions not regarded as parasitic by some workers have been found in a number of other diseases. In scarlet fever cellular inclusions occur in the skin lesons; in foot and mouth disease inclusions are found in the vesicles; in fowl pest inclusions are found in the brain and in epithelioma contagiosum or fowl diphtheria in the epithelium. Cytoplasmic inclusions accompanying many of the diseases of higher animals, and regarded as non-parasitic in most cases are not at all uncommon, but we have been unable to find any account in the literature of vertebrate diseases which are accompanied by the formation of *nuclear* inclusions as is the case with the polyhedral diseases of insects.

Bio-chemical Observations on the Polyhedral Bodies.

According to Tubeuf, Krassilschtschik, Prowazek, Wahl, Wolff, Glaser and Chapman the polyhedral bodies are regarded as being reaction products; towards bacteria (Tubeuf, Krassilschtschik) or towards Chlamydozoa (Prowazek, Wolff) or towards an unknown virus (Wahl) or towards a filterable virus (Glaser and Chapman). According to Bolle, Fischer, Marzocchi, and Knoche the polyhedral bodies are stages of a protozoan; according to Escherich and Miyajima they are bearers of an unknown virus.

It seems that the views are very diverse as regards the true nature of the polyhedra. For this reason it was thought advisable to submit some of our bio-chemical work on this subject in further support of our contention, obtained from morphological and experimental studies, that the polyhedra are merely organic degeneration-products of the disease.

During the gipsy moth season great quantities of diseased material can be obtained. For this reason all of the bio-chemical investigations were performed with gipsy moth polyhedra. These bodies are heavier than water and consequently can be obtained in bulk by centrifuging aqueous emulsions of diseased material. By repeated washing, filtering and centrifuging most of the fat, cellular débris, etc., can be eliminated. After this treatment the polyhedra were always washed with ether in order to free them from any possible remnants of adhering fat. Thorough attention to this cleansing operation will yield polyhedra in a fairly pure state for chemical tests. The material was allowed to dry naturally in the centrifuge tube, after which the lump that formed at the bottom could be loosened and transferred to a mortar where it was pulverized. This pulverization if done gently does not crack or injure the polyhedra in any way. After this procedure the mass of polyhedra look very much like pulverized chalk. It is comparatively easy to obtain 2 or 3 grams of polyhedra from about one or two hundred caterpillar cadavers.

As the polyhedra do not blacken with osmic acid, and do not stain with Sudan III., it seems unlikely that they contain fat. They stain with picric acid, however, and this gave us the clue to their possible protein nature. The color tests for dry proteins were then applied and we obtained positive reactions with xanthoproteic, Millon's, biuret, Adamkiewicz's and Lieberman's tests. It was next found necessary to obtain the polyhedra in solution so that the various coagulation or precipitation tests could be performed.

Before testing the solubility of the polyhedra in various reagents they were first rubbed energetically in an agate mortar with the addition of a little sea sand. This grinding was found necessary for the reason that the outer surface of the bodies is composed of more resistant material than the underlying strata. By grinding with sea sand the polyhedra are fragmented and this offers more delicate surfaces to the action of the reagents.

The polyhedra were found to be insoluble in hot or cold water, alcohol, chloroform, ether, or xylol. They dissolve readily in strong acids and alkalies, but these reagents were thought to produce too great a hydrolytic cleavage of the protein molecule, and since we did not wish to alter our material to any appreciable extent a number of milder reagents were tried. Moreover, from the standpoint of the classification of proteins it is important to determine just what will and what will not dissolve the material.

The following solubility tests were performed. Two grams of ground polyhedra were divided into four parts. To ½ gram water was added; to ½ gram .5 per cent. and to another ½ gram 2 per cent. NaCl solution, and to the fourth ½ gram 10 per cent. Na_2CO_3 were added. The tests were kept over a water bath for 13½ hours at a temperature varying between 55° and 58° C. At the end of this time the solutions were filtered and the various tests for soluble proteins applied. All of the tests (acetic acid, nitric acid, cupric sulphate, mercuric chloride, acetic acid with potassium ferrocyanide and ammonium sulphate) were negative showing that nothing went into solution.

Two grams of polyhedral material were again divided into four parts and treated respectively with H_2O, .5 per cent. and 2 per cent. NaCl and 10 per cent. Na_2CO_3. The tests were placed over a small direct flame for two hours. At the end of this time the solutions were filtered and the tests for soluble proteins applied. Negative tests were obtained with the water and salt solutions. The material treated with the 10 per cent. Na_2CO_3 solution gave slight coagulation tests with acetic and nitric acids showing that a small amount of protein went into solution. The polyhedra treated with the carbonate were examined microscopically and it was found that some fragments had partially dissolved while most of the polyhedra, which had apparently resisted fragmentation in the mortar had swollen to double their normal size.

Concentrated HCl (37 per cent.) used both hot and cold seems to dissolve the polyhedra with difficulty. The solubility of the bodies in boiling HNO_3 seems to lie between 15 and 20 per cent. We began with 4 per cent. HNO_3 in which the polyhedra are not affected and worked up to 31 per cent. HNO_3 in which they dissolve instantaneously on boiling. The fact that the liquid clears when some of the lower percentages of HNO_3 are used is not sufficient evidence that all of the polyhedra have been dissolved. For this reason the solubility of the bodies towards the acid was checked by microscopical examinations.

$(NH)_4OH$ does not seem to affect the polyhedra, but the other alkalies such as KOH and NaOH dissolve them readily. It was found that as low a percentage as 1/16 per cent. NaOH will dissolve polyhedra if they are boiled in the solution. For convenience 2 per cent. NaOH was used for the following tests. Two grams of ground bodies were dissolved in the alkali by means of heat. The solution was then dialyzed in order to get rid of the alkali. The dialysis was usually complete after 24 to 48 hours. At the end of this procedure the proteins remain in solution (*i. e.*, on dissolving in alkali, after which, although the alkali be removed, the polyhedra proteins remain soluble) and the tests for soluble proteins can be applied. We obtained positive reactions with acetic acid, nitric acid, cupric sulphate, mercuric chloride, acetic acid with potassium ferrocyanide and ammonium sulphate.

It might be well to mention that the percentages of NaOH used were accurate and the material pure. In making up the solution we did not rely on the so-called purity of the hydroxide sticks, but always eliminated every trace of Na_2CO_3 by precipitation with $Ba(OH)_2$.

After determining that the polyhedra are protein in nature it was next thought advisable to ascertain whether or not they are nucleoproteins. It seemed likely that this would be the case for the reason that they are formed in the nuclei of certain tissue cells. Two grams of whole (unrubbed) polyhedra were digested for one week in artificial gastric juice. After this time a microscopic examination failed to reveal any polyhedra. Theoretically, everything should have been decomposed excepting the nucleins which have a high phosphorus content. At the end of one week's digestion the material was filtered through and dried on ash free filter paper. The paper containing the residue was then cut into fine pieces and put into a platinum crucible with an oxidizing mixture (Na_2CO_3 (2 parts) + KNO_3 (1 part)). The material was slowly ignited and the residue dissolved in weak HNO_3. This solution was then warmed with the addition of some NH_4NO_3 in order to make the expected precipitate less soluble. Lastly 5 per cent. molybdic acid was added. The material was placed in an incubator and on standing a pronounced yellow precipitate (ammonium-phospho molybdate) was formed.

On the basis of this and the other tests described the polyhedra meet all of the requirements of the nucleoproteins. Nucleoproteins give all the color reactions, are soluble in water containing a small amount of alkali (1/16 per cent. in case of polyhedra) and are precipitated from this solution by acetic acid. The nucleins which have a high phosphorus content are not decomposed by gastric juice, and are obtained as an insoluble residue after the artificial digestion of nucleoproteins with pepsin.

Since iron is an element known to be contained in chromatin it was further thought advisable to determine whether the polyhedra during their synthesis from the chromatin and other substances in the nuclei embodied any iron. It will be needless to go through all the details of the analysis. Suffice it to say that every precaution to prevent iron contamination from water, air, etc., was used. Reagents known to be free from iron contamination were employed. Furthermore, the polyhedra were not rubbed with sand for fear of introducing iron in this manner. .0988 of a gram of polyhedra were used and .00049 of a gram of iron was found.

On dissolving polyhedra in alkali and after dialyzing away the alkali, three fractional precipitations with magnesium sulphate or sodium chloride can be obtained. Whether this means that the polyhedra are composed of three separate proteins or three groups of proteins, we are not prepared to say. The three precipitates, however, do demonstrate that the polyhedra are complex and not at all simple, a fact which does not seem strange when one reflects on the complexity of cellular or nuclear material in general.

As stated previously we regard the polyhedra as degeneration products formed during the course of the disease in the nuclei of certain tissue cells. The bodies are nucleoprotein crystal-like (pseudo-crystalline) aggregates. The idea suggested itself to us that it might be possible to dissolve the polyhedra and recrystallize them again after dissolution. If this should prove to be possible, it would militate seriously against the views held by Bolle, Fischer, Marzocchi, Knoche, Escherich and Miyajima that the bodies are organisms or the stages of an organism. Our experimental attempts at recrystallizing the polyhedra are a bit varied and so we do not wish to overemphasize our results as yet, but submit them with a full appreciation of their preliminary value.

Polyhedra were dissolved in 2 per cent. NaOH by heating. The process of dissolution was followed by an examination of samples microscopically, and when traces of the bodies could no longer be observed, the material was evaporated over a water bath and examined before it became entirely dry. Besides long Na_2CO_3 crystals we found many small and large bodies which resembled polyhedra. As a check we evaporated ordinary 2 per cent. NaOH, but we could not find the polyhedra-like crystals obtained with the protein solution. This seemed to be a result which offered possibilities, so we proceeded more carefully with another experiment.

Two grams of polyhedra were dissolved by heating in 2 per cent. NaOH. This solution was filtered and washed with ether in order to rid the material of any traces of fat. The ether was then eliminated by means of a separating funnel. The solution was next dialyzed to get rid of the alkali and a few protein tests were performed with some of the solution just to convince ourselves of the presence of proteins. This protein solution freed from the alkali was now slowly evaporated. On partial evaporation, we found beautiful single and double crystals which simulated the polyhedra very closely. If the material is evaporated completely it is difficult to find the crystals owing to the presence of coagulated and other protein material which hides them. If one shoots water under the cover-slip, however, the crystals again become visible just as soon as the coagulated sediment softens and becomes transparent. This fact that a coagulated residue remains shows that the entire protein material contained in the original polyhedra is not used during the formation of these new crystals. The majority of the crystals produced in this way simulate polyhedra very closely, but some are rounder and larger. Double forms are very common and are absolutely indistinguishable microscopically from ordinary polyhedra. The staining reactions of the crystals are similar to those of the polyhedra and Millon's reaction is identical. We have as yet not obtained a sufficient amount of these new crystals to submit them to all of the protein tests applied to the polyhedra. The crystals are not quite as stable as the polyhedra. They seem to lack the more resistant outer layer and therefore are more easily soluble in alkali and other reagents. For this and other reasons we do not claim to have reproduced typical polyhedra after their disintegration, but we firmly believe that the results are suggestive. It seems unreasonable, after submitting proteins to the violent hydrolytic action of both heat and alkali, to expect to reproduce the identical proteins. However, in the material under consideration, there seems to be a tendency for this particular protein or group of proteins to crystallize out in the shape characteristic of the polyhedra. These observations seem to support our view that the polyhedra are merely degeneration-products and not some inexplicable, unclassifiable organisms as supposed by many workers. An organism certainly could not be dissolved and its original form again reproduced or very nearly reproduced on evaporation.

So far we are unable to obtain the crystals after the dissolution of the polyhedra at every trial. Out of possibly ten trials, one usually succeeds four or five times. Undoubtedly some condition of which we are at present ignorant is responsible for the frequent failures. We have performed a sufficient number (15) of these recrystallization experiments, however, to warrant a report of the results.

Crystallizable proteins are, of course, not uncommon. Hæmoglobin is perhaps the best known example in animals and the aleurin grains a well known example in plants. By fractional precipitation with magnesium sulphate or sodium chloride two or three separate crystalline proteins can be obtained from the albumen of the hen's egg. In insects, by the evaporation of blood with a trace of acetic acid, beautiful protein crystals can be obtained, different in every species.

Our view regarding the nature of the polyhedral bodies may therefore be summarized as follows: During the course of the disease the virus disintegrates the nuclear material in such a way that crystal-like bodies called polyhedral bodies or polyhedra are synthesized out of the disintegrating proteins. Just how the process from nuclear material to polyhedra takes place is at present unknown. At any rate, from our morphological observations, experimental infection data (published elsewhere) and from our chemical studies here presented, it seems clear that the polyhedra are nucleoprotein degeneration-products and not organisms responsible for a series of insect diseases.

Summary.

1. Polyhedral bodies are found in many different species of lepidopterous larvæ.
2. The bodies are specific for a certain type of disease.
3. The polyhedra vary in size in the different species.
4. There exists a striking similarity in shape between the polyhedra found in different species.
5. The polyhedra are structurally complicated.
6. They arise in the nuclei of certain tissue cells.
7. Cytoplasmic inclusions are found in certain diseases of higher animals.
8. Nuclear inclusions have not been known previously.
9. The polyhedra are nucleoprotein crystal-like degeneration-products and not organisms.

10. The polyhedra contain iron and phosphorus.

11. On dissolving polyhedra in alkali and after dialyzing away the alkali and evaporating the protein solution crystals are obtained which simulate the original polyhedra.

BIBLIOGRAPHY.

Chapman, J. W. and Glaser, R. W.

'15 A Preliminary List of Insects which Have Wilt, with a Comparative Study of their Polyhedra. Journal Econ. Ent., Vol. 8, No. 1, pp. 140–149, 1 pl.

'16 Further Studies on Wilt of Gipsy Moth Caterpillars. Journal Econ. Ent., Vol. 9, No. 1, pp. 149–167. Tables 21.

Escherich, K. and Miyajima, M.

'11 Studien über die Wipfelkrankheit der Nonne. In Naturw. Ztschr. Forst u-. Landw. Jahrg. 9, Heft 9, s. 381–402, 6 fig.

Glaser, R. W. and Chapman, J. W.

'13 The Wilt Disease of Gipsy Moth Caterpillars. Journal Econ. Ent., Vol. 6, No. 6, pp. 478–488.

Glaser, R. W.

'14 The Bacterial Diseases of Insects. Psyche, Vol. 21, No. 6, pp. 184–190.

'15 Wilt of Gipsy Moth Caterpillars. Journal of Agri. Research, Vol. 4, No. 2, pp. 101–128, 17 fig., 4 pl.

Huntemüller

'13 Filtrierbare Virusarten. Ztschr. f. Chemotherapie, Bd. 2, Heft 1, pp. 56–69, Tafeln 9.

Osborne, Thomas B.

'99 Crystallized Vegetable Proteids. Am. Chem. Jour., Vol. 14, No. 8, pp. 1–28, fig. 3.

'99 Egg Albumin. Jour. of Am. Chem. Society, Vol. 21, No. 6, pp. 477–485

'99 On Some Definite Compounds of Protein-bodies. Jour. of Am. Chem. Society, Vol. 21, No. 6, pp. 486–493.

Osborne, Thomas B., and Campbell, George F.

'00 The Proteids of the Egg Yolk. Twenty-third Report of the Conn. Agri. Ex. Sta., pp. 339–348.

'00 The Protein Constituents of the Egg White. Twenty-third Report of Conn. Agri. Ex. Sta., pp. 348–375.

Osborne, Thomas B., and Harris, Isaac F.

'03 The Precipitation Limits with Ammonium Sulphate of Some Vegetable Proteins. Jour. of Am. Chem. Society, Vol. 25, No. 8, pp. 837–842.

Prowazek, S. von.

'07 Chlamydozoa. II. Gelbsucht der Seidenraupen. In Arch. Protistenk., Bd. 10, Heft 2/3, s. 359–364, 2 fig.

'12 Untersuchungen über die Gelbsucht der Seidenraupen. In Centbl. Bakt. (etc.), Abt. 1, Orig., Bd. 67, Heft 4, s. 268–284, 1 fig., 2 pl.

'12 Gelbsucht (Polyederkrankheit) der Raupen. In Prowazek's Handbuch der Pathogenen Protozoen, Bd. I., s. 156–161, 2 fig., Verlag von Johann Barth. Leipzig, 1912.

Tubeuf, Carl von.

'92 Die Krankheiten der Nonne. (Liparis monacha.) In Forstl. Naturw. Ztschr., Jahrg. 1, Heft 1, s. 34–37, fig. 9–16; Heft 2, s. 62–79.

'92 Weitere Beobachtungen über die Krankheiten der Nonne. In Forstl. Naturw. Ztschr., Jahrg. 1, Heft 7, s. 277–279.

'11 Zur Geschichte der Nonnenkrankheit. In Naturw. Ztschr. [Forst u-. Landw., Jahrg., 9, Heft 8, s. 357–377.

Wolbach, S. B.

'12 The Filterable Viruses, a Summary. Jour. of Med. Research, Vol. 27, No. 1 (New Series, Vol. 22, No. 1), pp. 1–25.

'11 The Nature of Trachoma Bodies. Jour. Med. Research, N. S., Vol. 19, No. 2, pp. 259–264. 3 pl.

Wolff, M.

'10 Ueber eine neue Krankheit der Raupe von Bupalus piniarius L. In mitteilung des Kaiser Wilhelm Institutes für Landwirtschaft in Bromberg, Bd. III, Heft 2, s. 69–92.

Wahl, Bruno.

'09–'12 Ueber die Polyederkrankheit der Nonne (Lymantria monacha L.). In Centbl. Gesam. Forstw., Jahrg. 35, Heft 4, s. 164–172; Heft 5, s. 212–215; Jahrg. 36, Heft 5, s. 193–212; Heft 8/9, s. 377–397; Jahrg. 37, Heft 6, s. 247–268; Jahrg. 38, Heft 8/9, s. 355–378.

PLATE I.

FIG. 1. Photomicrograph of a smear showing free polyhedra.

FIG. 2. Photomicrograph showing various stages during the formation of polyhedra in tissue nuclei of a gipsy moth caterpillar. × 720.

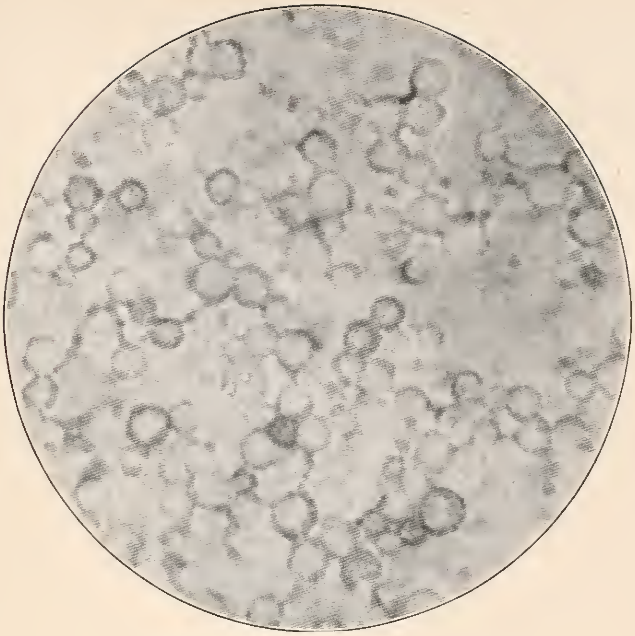


FIG. 1.

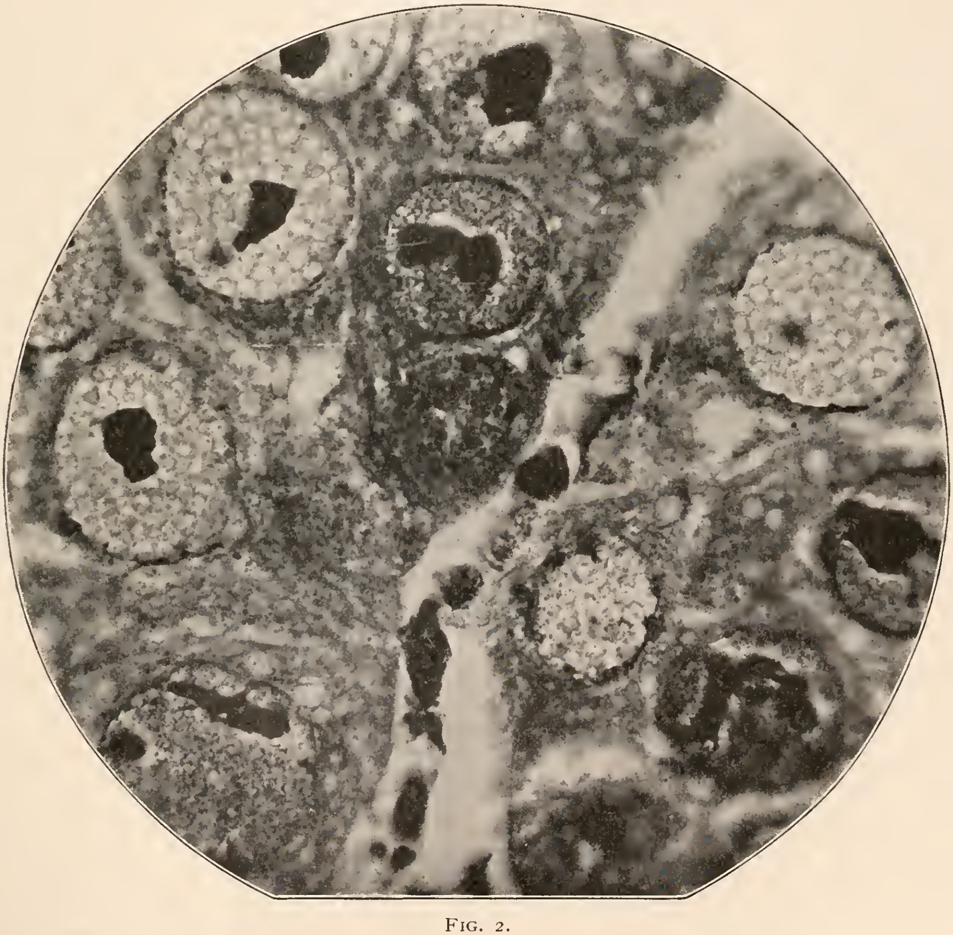


FIG. 2.

R. W. GLASER AND J. W. CHAPMAN.

Plate II.

Fig. 3. Photomicrograph of army worm tissue showing fully developed polyhedra in nuclei of fat cells. × 300.

Fig. 4. Photomicrograph of army worm tissue showing normal hypodermal, fat, muscle and blood cells. × 300.

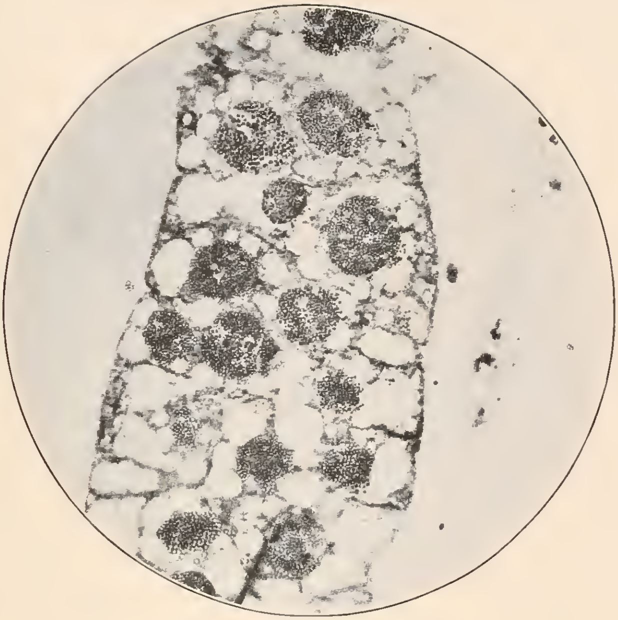


FIG. 3.

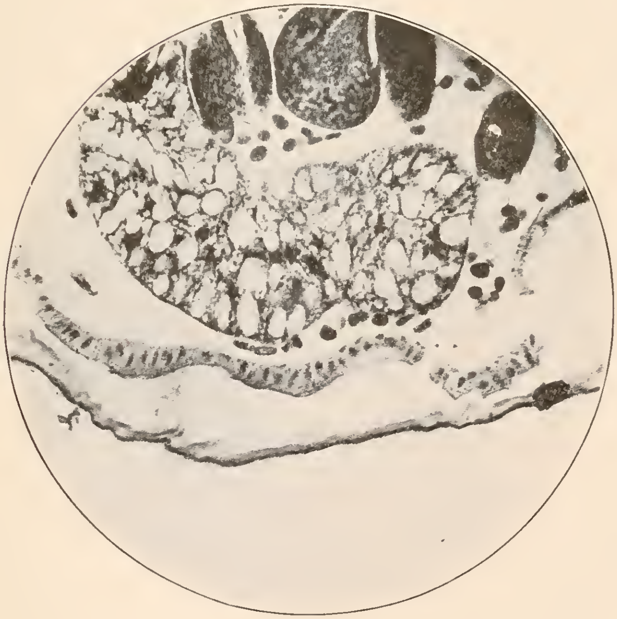


FIG. 4.

PLATE III.

FIG. 5. Photomicrograph of army worm tissue showing normal, hypodermal, fat, muscle and blood cells. × 300.

FIG. 6. Photomicrograph of army worm tissue showing disintegration of nuclei and cells with liberation of polyhedra. × 300.

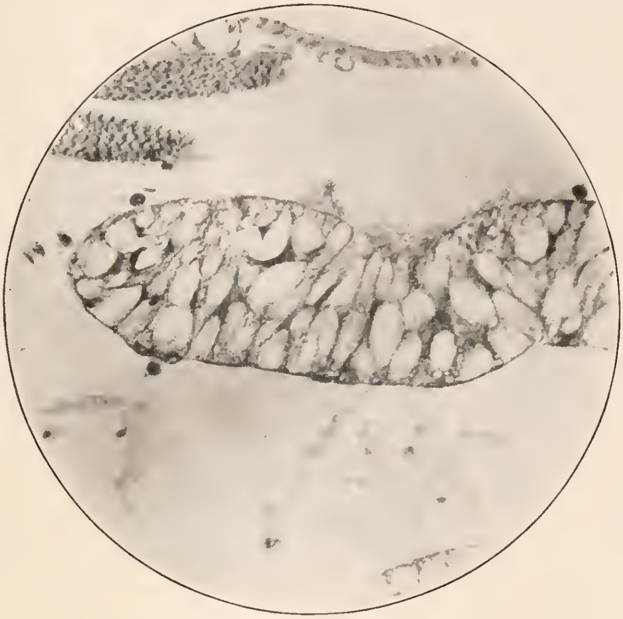


FIG. 5.

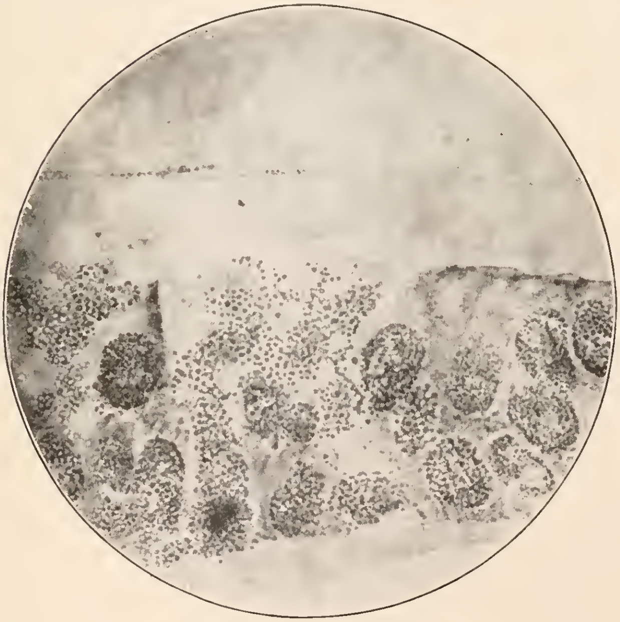


FIG. 6.

AXIAL SUSCEPTIBILITY GRADIENTS IN THE EARLY DEVELOPMENT OF THE SEA URCHIN.

C. M. CHILD.

(WITH 20 FIGURES.)

Axial gradients in susceptibility to cyanides and various other agents have already been demonstrated in *Planaria* (Child, 13*b*) various infusoria (Child, '14), the early developmental stages of the starfish (Child, '15*a*), in a number of species of Oligochetes (Hyman, '16), in a number of algæ and in various other animals the data for which are as yet unpublished. Thus far such gradients have been found, at least in the earlier stages of development in all forms examined, comprising more than sixty species and including algæ, cœlenterates, flatworms, echinoderms, annelids, and vertebrates. The relation of these gradients to developmental gradients of other kinds and the problem of their significance for the physiological individual has also been considered (Child, '15*b*, Pt. III, '15*c*).

During the summers of 1913 and 1915 at the Marine Biological Laboratory, Woods Hole, Mass., axial susceptibility gradients were demonstrated in the early developmental stages of the sea urchin, *Arbacia punctulata*, and the control and modification of development by means of the differential susceptibility along these gradients was found to be possible. In the present paper only the direct evidence for the existence of such gradients, based on the progress of death and disintegration along the axis, is considered and this is incomplete in certain respects. The indirect evidence from the control and modification of development, with which I was chiefly concerned and which is of greater interest, will be presented in another paper.

METHODS OF DEMONSTRATING SUSCEPTIBILITY GRADIENTS.

The method employed in demonstrating the gradients has already been described (Child, '13*a*, '15*b*, Chap. III.) and consists in directly determining the differences in susceptibility along the axis or axes to cyanides and various other agents used in concentrations sufficient to kill in the course of a few hours, but not high enough to kill immediately and not low enough to permit the organisms to become acclimated or acquire a tolerance to them. The agents used in these studies on the sea urchin were potassium cyanide, ammonium hydrate, ethyl alcohol and hydrochloric acid in sea water. The various developmental stages were placed in concentrations of these substances determined by preliminary experiment and the progress of death along the axis was observed.

In many of the lower animals the progress of swelling, cytolysis, separation and disintegration serves directly as an indication of the progress of death. In the developmental stages of the sea urchin changes of this sort occur as the cells die, but they differ somewhat with different reagents and different stages of development. In KCN the cells swell, become spherical, and separate from each other as they die and the region concerned breaks down into a shapeless mass of these spherical cells which soon disintegrate. If motor activity is still present in other parts the dead cells may be progressively left behind as the living portion moves about. This disintegration of the body is more marked in the earlier stages of the blastula and gastrula than in later stages where supporting tissues have differentiated, but even in the later stages extensive disintegration can be brought about by return to sea water after a sufficient length of time in KCN. These death changes are somewhat accelerated and intensified by the return to water and death is marked by very complete disintegration. Thus when KCN is used, the progress of death can either be followed directly under the microscope in the KCN solution, at least in the blastula and gastrula stages, or lots may be returned to water at stated intervals and the progress of death determined by the comparison of dead and living portions of the body in successive lots. Both methods have been used, but the latter is more satisfactory in many cases, because after return to water the dead portions disintegrate rapidly and completely while the parts which are still alive may recover and resume motor activity where the stages in which movement occurs are concerned. The progress of death can also be made visible by staining with neutral red before placing in KCN. When death occurs the neutral red color changes to yellow, as the alkali of the KCN solution penetrates the cells, and then disappears.

The death changes in ammonium hydrate are very similar to those in KCN. The cells swell and in the blastula and gastrula stages separate, but in later stages the cells cohere more or less after death and the visible death changes are merely swelling and rounding of the cells and a consequent increase in size and greater translucency of the whole. Here likewise, return to water increases the disintegration and so makes it easier to follow the progress of death, and neutral red may also be used as an indicator of death in the same way as with KCN.

Ethyl alcohol is used in the same way as KCN and NH_4OH, but of course in much higher concentration. Disintegration of blastula and gastrula stages is very complete and in the plutei only the supporting tissues retain the body form.

In hydrochloric acid, however, the behavior of the cells is different, as might be expected. The cells shrink and do not separate and death may occur with very little visible change except in size. But return to sea water after a sufficient length of time in HCl brings about disintegration and the dead and dying cells swell and separate so that the progress of death can be followed without difficulty by removing lots from HCl to sea water at regular intervals.

As regards concentration of the reagents used, a wide range is possible according to the stage of development and the length of survival time desired. Since the susceptibility increases very greatly from fertilization up to the blastula stage as physiological rejuvenescence occurs (Child, '15*b*, pp. 412–418), much higher concentrations can be used for the former than for the latter stages. At a temperature of 22–24° C. unfertilized eggs begin to die after 4–5 hours in KCN *m*/100, while blastulæ and later stages begin to die after the same length of time in KCN *m*/1,000. The other reagents were used only on the blastula and later stages. In NH_4OH *m*/500 death of these stages begins in half an hour to an hour, in *m*/1,000 in 1–2 hours. The same stages in alcohol 4 per cent. (roughly *m* 2/3) begin to die in 3–4 hours and after 5–10 minutes in HCl $m/400$ death begins on return to water, but it is difficult to determine just when it occurs, if the stages are left in HCl. Much lower concentrations of KCN and NH_4OH can be used without the occurrence of any appreciable degree of acclimation, but acclimation to alcohol and HCl takes place much more rapidly and in relatively high concentrations, alcohol 1.5 per cent., HCl $m/2{,}000$, so that for direct demonstration of the susceptibility gradient with these reagents the range of concentration is not so great.

The significance of differences in susceptibility to cyanides, various narcotics and certain other agents has been considered elsewhere (Child, '13*a*, '15*b*, Chap. III.). It has been found that in concentrations high enough to kill without acclimation the susceptibility varies in general directly with the rate of metabolism or of certain fundamental reactions, while in concentrations low enough to permit acclimation the higher the metabolic rate the greater the degree and rapidity of acclimation consequently in the long run the susceptibility to these concentrations varies inversely as the metabolic rate.

Although the susceptibility method has proved in certain cases to be a very satisfactory means of distinguishing differences in general metabolic activity, it is actually of course a rather crude method and merely makes it possible to compare in a rough way metabolic differences in different individuals or body regions and does not of course give us any exact quantitative data. Moreover, the nature of the method makes it evident that we cannot expect to distinguish with certainty the minuter metabolic differences, because the reagents used decrease to some extent the differences which they are expected to show. Definite and constant differences in susceptibility must mean considerable differences in rate of reaction, but the absence of such differences in susceptibility does not necessarily mean the complete absence of differences in rate of reaction. Notwithstanding these limitations, however, the method is useful and the positive results obtained by means of it afford ample proof of its value.

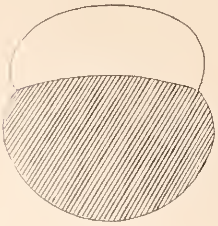
One further point requires some consideration. The method has been criticized, more particularly in personal conversation, as involving certain assumptions concerning the action on living chemically active protoplasm of cyanides and other agents used. This, however, is not the case. There is no reason for believing that different agents which retard or inhibit metabolic activity in living protoplasm all act in the same way. Protoplasm is a complex system in which both the physical substratum and the chemical reactions play a part, but the important point is that it is a system, *i. e.*, that the different processes, changes and conditions in it are not independent of each other but mutually correlated and dependent to a greater or less degree. It is not in the least improbable that different agents may give the same general results, as regards susceptibility, even though one acts primarily on the aggregate condition of the colloids or let us say the permeability of membranes, another on the production or constitution of enzymes, and still another on the chemical reactions of oxidation. My observations on susceptibility to cyanides, various narcotics, acids, alkalies, metabolic products, and even temperature have convinced me that the relation between susceptibility to retarding or inhibiting agents and conditions and general metabolic rate or the rate of certain fundamental metabolic reactions is a very general relation and there seems to be absolutely no reason for believing that it is dependent upon any one particular method of action on the protoplasmic system of the agent or condition employed. It still remains of course for future investigation to determine the exact method of action of each agent and condition, to formulate the general rule and to discover and account for exceptions if they exist. Since our present knowledge indicates both that the oxidations are fundamental metabolic reactions and that they are more or less dependent on various conditions in the protoplasmic system; we may expect to find that their rate is altered by a great variety of external agents and conditions even though these do not enter directly into the chemical reactions of oxidation.

In certain cases, as in the green plants, the energy for certain of the synthetic or anabolic reactions is derived directly from sources outside the organism and in such cases these reactions may be to a considerable degree independent of the energy-producing reactions in the organism. Such reactions, however, are in a sense only preliminary, although of course essential to the fundamental processes of life and the relation between susceptibility and metabolic rate is primarily concerned, not with them, but rather with the reactions which play a fundamental rôle in setting free the energy characteristic of living organisms.

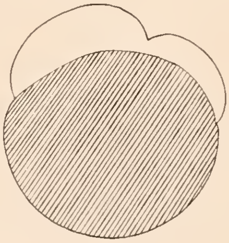
In the sea urchin the differences in susceptibility along the axis so far as observed, are essentially the same whether cyanides, alcohol, alkalies or acids are used as reagents. All these substances interfere with the action of the protoplasmic system in some way, but by no means necessarily in the same way, yet the general results as regards susceptibility are the same. This fact is highly significant and indicates to some extent the general character of the relation between susceptibility and the fundamental conditions and processes of life.

The Eggs and Cleavage Stages.

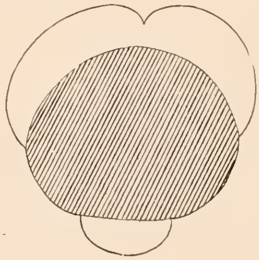
Since experiments on the control and modification of development through differential susceptibility along the axes had shown very conclusively the existence of axial gradients in the early developmental stages and had indicated their probable presence even in the unfertilized egg, and since it was soon found that the character of the death changes made it difficult to reach definite conclusions concerning death gradients from direct observation, but little time was spent on these earlier stages and the evidence obtained, so far as it has any value, is merely contributory. I regret to state, however, that I neglected to employ as a means of orientation the method of making the micropyle visible by colored suspensions in the water. This might have made the data on these earlier stages somewhat more conclusive.



1

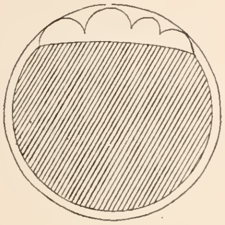


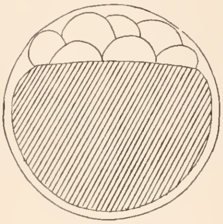
2

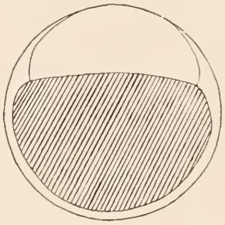


3

The unfertilized eggs in KCN $m/100$ show death changes of the character indicated in Figs. 1–3. First, one or more clear droplets or masses which contain some colloids and much water appear on the surface of the egg and grow larger, while the granular portion decreases in size. This process may go on, the clear masses often uniting, until the clear and granular portions are almost equal in size, but finally the granules spread and the distinction between clear and granular areas disappears. The only indication of a susceptibility gradient is the appearance of the clear droplets only or earlier on one hemisphere or at one pole. Within certain limits of concentration this has been observed in some 70–80 per cent. of the eggs, but in some eggs the clear droplets appear at opposite poles or over various parts of the surface at the same time. The fertilized eggs before cleavage (Figs. 4–6) behave in KCN essentially like the unfertilized.

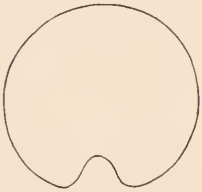

4

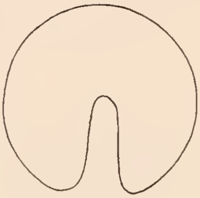

5

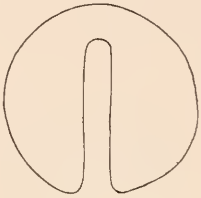

6

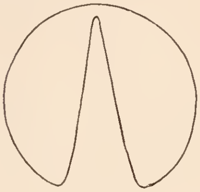
The blastomeres of the earlier cleavages show a similar separation into clear and granular areas and here again the clear areas or droplets usually appear first or only on one hemisphere or at one pole of the egg. In eggs placed in KCN after the appearance of the micromeres I believe that the apical hemisphere is in general more susceptible than the basal.

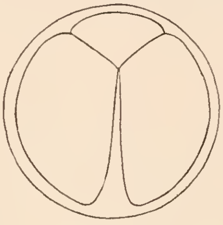
An interesting gradient in the first cleavage appears in certain concentrations of cyanide. In such cases the first cleavage plane at its first appearance extends as a furrow only half way or less around the egg and cuts through the egg in one direction without the appearance of any furrow on the opposite side (Figs. 7–10). In some cases a larger or smaller portion of the cytoplasm is separated from the two blastomeres at the end of this cleavage by the division of the cleavage furrow into two (Fig. 11). Such cleavages occurred in about 40 per cent. of eggs placed in KCN *m*/1,000 for 10½ hours before fertilization then well washed and kept in sea water and fertilized one hour after removal from KCN. Figs. 7–10 are drawn from such eggs. After this treatment there is no elevation of a fertilization membrane from the egg surface, and the blastomeres frequently become entirely separated after cleavage. Eggs placed in KCN *m*/100 fifteen minutes after fertilization and washed and returned to sea water after three hours also showed cleavage of this kind in 30–40 per cent. In the other eggs of these lots cleavage is normal or incomplete and often irregular or else delayed with simultaneous formation of a number of blastomeres. Apparently then these one-sided cleavages represent the first step in departure from normal cleavage. The indirect evidence shows that a susceptibility gradient is present in these stages of development and that the region of highest susceptibility and therefore of highest metabolic rate is the apical pole. It is evident that cyanide inhibits cleavage, and if the apical pole is most susceptible, we should expect to find cleavage most completely inhibited there, and least inhibited at the basal pole. It is probable, therefore, that these cleavage furrows start from the basal and proceed toward the apical pole,


7


8


9


10


11

and that in cases like that in Fig. 11, where a portion of the cytoplasm is cut off, it is the apical region, which is so much injured that it cannot give rise to a cleavage furrow.

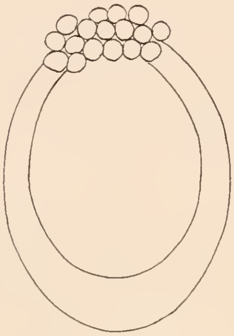
With the same methods of treatment a general gradation in the size of the blastomeres was observed in 30–40 per cent. of the eggs in stages from 32 cells onward, though it became less marked in later stages as recovery proceeded. In these cases also it is probable that the region of most rapid cleavage and therefore of the smallest blastomeres is the basal region and the region where cleavage is most inhibited and therefore the blastomeres are largest is the apical region.

The small size and protoplasmic character of the micromeres at the basal pole suggests the possibility that their metabolic rate may be higher than that of the cells about them, but I have not been able to discover that their susceptibility is greater than that of the adjoining cells, and the indirect evidence shows that the mesenchyme cells, which are believed to be at least in part descendants of the micromeres are among the least susceptible if not the least susceptible of all the cells.

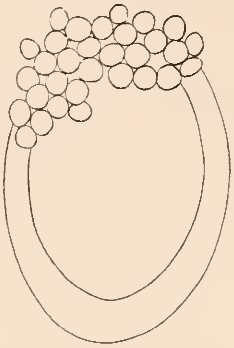
These observations on the earlier developmental stages are not conclusive. Taken by themselves they are of little value, but considered in the light of the indirect evidence and of the more definite results obtained in later stages they possess a certain significance as contributory evidence. My observations on these stages were only incidental to other work and only KCN was used as reagent.

The Blastula and Gastrula.

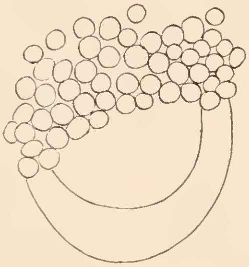
As soon as the blastula begins to elongate the direction of movement, and in more advanced stages the greater thickness of the cellular wall in the basal region make it possible to distinguish apical and basal ends without difficulty. In these stages death begins at the apical end and proceeds basally (Figs. 12–15), advancing in many cases somewhat more rapidly down one side (Figs. 13, 14), probably that side which later becomes the anterior end of the pluteus. The progress of death in the basal direction is very regular, though a few cells here and there may swell and push out of the body-wall earlier than others about them. The susceptibility gradient is the same with all the reagents and methods of use noted in the preceding section so that there can be no doubt of the existence of an apico-basal gradient which is fundamentally related to the activity of the living protoplasm. The more rapid progress of death down one side of the blastula which is frequently observed is probably an indication of differences in other axes, but on this point certainty is impossible.



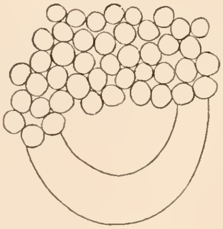
12



13



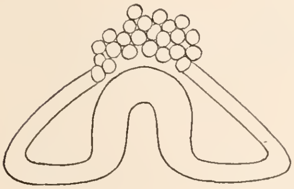
14



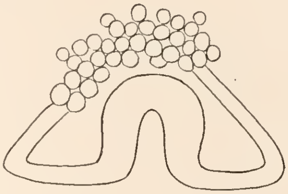
15

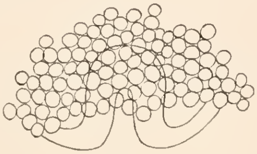
In the gastrula stage the same gradient appears (Figs. 16–18), the apex of the gastrula, which represents the apical region of the egg and blastula, being most susceptible, the basal least susceptible. The susceptibility of the entoderm and the blastopore region is very much lower than that of the other ectodermal regions. The entoderm is still intact after practically the whole ectoderm has disintegrated (Fig. 18) and in concentrations where ectodermal disintegration occurs in three or four hours disintegration of the entoderm is usually not complete until one or two hours later or in some cases even a longer time.

By returning to sea water after the proper length of time in the reagent it is possible to stop death at any level of the body and to bring about recovery and further development of the parts still alive. In the blastula it is more or less of the basal portion which remains alive, and this may close up and gastrulate, producing dwarf gastrulæ with a disproportionately large enteron. In the gastrula stage also partial dwarf gastrulæ with large enteron result from partial disintegration and recovery, because the more apical ectoderm may be in large part destroyed while the basal region of the gastrula and the entoderm remain intact. The forms of larvæ which develop from these partial basal blastulæ and gastrulæ will be described in another paper.



16



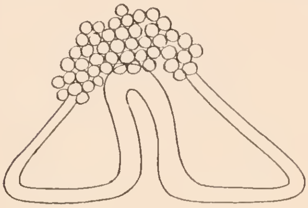


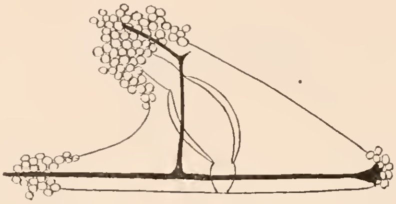
18

Later Stages.

In the early stages of transformation into the pluteus, the gastrula loses its apparent radial symmetry and becomes triangular in outline in basal or anal view, the base of the triangle representing the anterior region of the future pluteus. In side view the apex of the gastrula is seen to be shifted toward the anterior end as compared with earlier stages (*cf.* Figs. 19 and 16). In the further transformation this apical region becomes the oral lobe of the pluteus and the long anal arms develop from the basal region of the anterior end (Fig. 20).

The susceptibility gradient in the earlier stages of this transformation is the same as in the gastrula, apico-basal (Fig. 19) and the same difference in susceptibility between ectoderm and entoderm persists. Later, when the larva begins to elongate in the antero-posterior direction, and the anal arms begin to develop, these arms and the posterior end both appear as secondary regions of high susceptibility (Fig. 20) though the susceptibility of the anal arms is in general somewhat less than that of the oral lobe and that of the posterior end somewhat less than that of the anal arms. On the oral lobe and over the body death progresses in the basal and posterior direction and in the anal arms from tip to base of the arms.


19


20

In the fully developed pluteus the susceptibility gradients are less marked. The ectoderm of the oral lobe and anal arms is still somewhat more susceptible than that of other regions but the differences are less conspicuous. In all these later stages, however, the entoderm remains much less susceptible than the ectoderm and apparently the mesenchyme is least susceptible of all parts.

It is probable that the gradual fading out of the metabolic gradients in the pluteus is a physiological change which precedes and makes possible the development of the axial gradients of the mature sea urchin which have been previously inhibited by the existing axial relations. If this suggestion is correct these changes are the factors which determine, or rather permit metamorphosis.

The development of the arms and the posterior elongation has been shown to be dependent on the development of the skeleton. That being the case the appearance of high susceptibility in the anal arms and the posterior end is not self-determined in these parts but results from skeletal growth.

Discussion and Summary.

The existence in the apico-basal axis of the blastula, gastrula and later stages of a definite and conspicuous gradient in susceptibility which is the same for cyanide, alcohol, acid and alkali is a significant fact, particularly in the light of the relations between susceptibility to cyanides and various narcotics and general metabolic rate. The results with HCl and NH_4OH show that this relation is the same for these substances. The further evidence for the existence of this gradient in the earlier developmental stages, together with the difficulty of conceiving how an apico-basal gradient could arise *de novo* during the earlier development, leave little doubt that this axial gradient persists from the unfertilized egg to the mature pluteus. This conclusion will be confirmed by the indirect evidence presented elsewhere.

As regards gradients in other axes the evidence is less conclusive. It is probable that the asymmetry in the progress of death frequently observed in the blastula (Figs. 13, 14) is an indication of a difference between anterior and posterior regions and this probability is strengthened by the appearance of a similar asymmetry in the gastrula and prepluteus stages (Figs. 17 and 19).

It must be remembered, however, that this method of determining susceptibility is far from being a perfect method for the demonstration of differences in metabolic rate. It can be expected to show only the grosser differences, for the reagents used tend to decrease the differences which they are used to demonstrate, and if the differences are originally slight they may disappear so rapidly in the reagent that no appreciable or constant differences in the time of death appear.

It is evident from the course of development that the apico-basal gradient is the most strongly marked and in the early stages even this is not very clearly defined by differences in susceptibility as determined by time of death, though it becomes more distinct later. It is not to be expected that the minor differences along the axes of symmetry should appear as clearly by this method as the differences along the major axis. The important facts are that the major axis appears so distinctly as a susceptibility gradient and that indications of susceptibility differences in the minor axes have been observed.

The apico-basal susceptibility gradient is the same as that in the starfish (Child, '15*a*) and in all other forms examined, at least in early developmental stages and in many cases throughout life. That this gradient is of fundamental significance cannot be doubted and I have attempted elsewhere (Child, 15*c*) to present some of the evidence which seems to me to indicate that a physiological axis is fundamentally such a gradient in metabolic rate.

The chief points are summarized as follows:

1. A distinct gradient in susceptibility to potassium cyanide, ethyl alcohol, ammonium hydrate and hydrochloric acid is present along the apico-basal axis of blastula, gastrula, and later stages of larval development of *Arbacia* and indications of a gradient are found in earlier stages.

2. In the apico-basal gradient the susceptibility is highest at the apical end of the axis and lowest at the basal end. Since susceptibility to these reagents varies in general directly with metabolic rate, the susceptibility gradient indicates the existence of a gradient in rate of metabolic activity in which the rate is highest in the apical region and decreases basally.

3. Some indications of gradients in other axes appear in the differences of susceptibility, but these are much less distinct than the differences along the apico-basal axis.

4. The anal arms and the posterior end of the larval body appear as secondary regions of high susceptibility after they begin to develop.

5. In the fully developed pluteus these susceptibility gradients become less marked and doubtless disappear as metamorphosis begins.

HULL ZOOLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
February, 1916.

REFERENCES.

Child, C. M.

13a Studies on the Dynamics of Morphogenesis and Inheritance in Experimental Reproduction. V. The Relation Between Resistance to Depressing Agents and Rate of Reaction in Planaria dorotocephala and its Value as a Method of Investigation. Jour. Exp. Zool., XIV.

'13b Studies, etc. VI. The Nature of the Axial Gradients in Planaria and their !Relation to Antero-posterior Dominance, Polarity and Symmetry. Arch. f. Entwickelungsmech., XXXVII.

'14 The Axial Gradient in Ciliate Infusoria. BIOL. BULL., XXVI.

'15a Axial Gradients in the Early Development of the Starfish. Amer. Jour. Physiol., XXXVII.

'15b Senescence and Rejuvenescence. Chicago.

'15c Individuality in Organisms. Chicago.

Hyman, L. H.

'16 An Analysis of the Process of Regeneration in Certain Microdrilous Oligochetes. Jour. Exp. Zool., XX, 2.

Vol. XXX. *June, 1916.* *No. 6*

BIOLOGICAL BULLETIN

THE MARINE BIOLOGICAL LABORATORY

EIGHTEENTH REPORT; FOR THE YEAR 1915

TWENTY-EIGHTH YEAR.

I. TRUSTEES (AS OF AUGUST, 1915) 407
II. ACT OF INCORPORATION 408
III. BY-LAWS OF THE CORPORATION 409
IV. THE TREASURER'S REPORT 411
V. THE LIBRARIAN'S REPORT 415
VI. THE DIRECTOR'S REPORT 418
  Statement 418
  1. The Staff 422
  2. Investigators and Students 425
  3. Tabular View of Attendance 431
  4. Subscribing Institutions 431
  5. Evening Lectures 432
  6. Members of the Corporation 433

I. TRUSTEES

EX OFFICIO

FRANK R. LILLIE, *Director*, The University of Chicago.

GILMAN A. DREW, *Assistant Director and Vice-President*, Marine Biological Laboratory.

D. BLAKELY HOAR, *Treasurer*, 161 Devonshire Street, Boston, Mass.

GARY N. CALKINS, *Clerk of the Corporation*, Columbia University.

TO SERVE UNTIL 1918

CORNELIA M. CLAPP..... Mount Holyoke College.
E. G. CONKLIN.......... Princeton University.
ROSS G. HARRISON....... Yale University.
CAMILLUS G. KIDDER..... 27 William Street, New York City.

M. M. Metcalf	Oberlin, Ohio.
William Patten	Dartmouth College.
Jacob Reighard	University of Michigan.
W. B. Scott	Princeton University.

TO SERVE UNTIL 1917

S. F. Clarke	Williams College.
Charles A. Coolidge	Ames Building, Boston, Mass.
C. R. Crane	Woods Hole, Mass., *President of the Board.*
Alfred G. Mayer	Carnegie Institution.
C. E. McClung	University of Pennsylvania.
T. H. Morgan	Columbia University.
Erwin F. Smith	United States Department of Agriculture.
E. B. Wilson	Columbia University.

TO SERVE UNTIL 1916

H. H. Donaldson	Wistar Institute of Anatomy and Biology.
M. J. Greenman	Wistar Institute of Anatomy and Biology.
C. W. Hargitt	Syracuse University.
H. S. Jennings	Johns Hopkins University.
George Lefevre	University of Missouri, *Secretary of the Board.*
A. P. Mathews	The University of Chicago.
G. H. Parker	Harvard University.
Henry B. Ward	University of Illinois.

TO SERVE UNTIL 1915

H. C. Bumpus	Tufts College.
R. A. Harper	Columbia University.
W. A. Locy	Northwestern University.
Jacques Loeb	The Rockefeller Institute for Medical Research.
F. P. Mall	Johns Hopkins University.
George T. Moore	Missouri Botanical Garden, St. Louis.
L. L. Nunn	Telluride, Colo.
John C. Phillips	299 Berkeley Street, Boston, Mass.

II. ACT OF INCORPORATION

No. 3170.

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, Henry B. Pierce, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our LORD ONE THOUSAND, EIGHT HUNDRED and EIGHTY-EIGHT.

HENRY B. PIERCE,
Secretary of the Commonwealth.

[SEAL.]

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio*, members of the Board of Trustees, and Tustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio*.

II. Special meetings of the members may be called by the Trustees, to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee on finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution, on such terms as may then be agreed upon.

IX. These By-Laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-Laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. TREASURER'S REPORT

Inasmuch as the records of the Laboratory have been reorganized on an income and expense basis, taking into account the bills receivable and payable, as well as the cash receipts and disbursements, the treasurer makes a departure from the form of report previously employed in order to be in accord with the new methods.

The cash statement of operations is, therefore, now presented in consolidated form and the income and expense schedule follows accompanied by a balance-sheet, showing the condition of the Laboratory on December 31, 1915, as far as the figures are available. Figures for the plant and inventory valuations are in process of compilation.

Consolidated Cash Statement of Receipts and Disbursements for the Year ended December 31, 1915

Operating Accounts

Cash on hand January 1, 1915			$1,686.68
Receipts from departments		$46,676.99	
Receipts from donations		35,500.00	
Receipts from work done for others		1,402.16	
Receipts from loans repaid		125.00	
Total receipts for year		$83,704.15	
Payments for departmental expenses	$61,856.38		
Payments for improvements	16,863.51		
Payments for work done for others	1,537.05		
Payments for new town landing	761.50		
Payments for Loan	25.00		
Total payments		81,043.44	
Excess of receipts for year			2,660.71
			$4,347.39
Less Oklahoma warrant included in cash receipts but not yet paid			135.23
Cash balance December 31, 1915			$4,212.16

CASH RECEIPTS AND PAYMENTS ON ACCOUNT OF INVESTMENTS FOR THE YEAR ENDED DECEMBER 31, 1915

RESERVE FUND

Cash on hand January 1, 1915	$210.30	
Receipts:		
Interest —$3,000 American Telephone & Telegraph Company, 4 per cent bonds	60.00	
¾ of $500 Western Telephone & Telegraph Company 5's	18.74	
Dividend—6 shares American Smelting & Refining Company, preferred	42.00	
8 shares General Electric Company	64.00	
14 shares United Shoe Machinery Corporation, preferred	21.00	
2 shares Massachusetts Gas, preferred	8.00	
Interest on bank deposit	1.04	
	$425.08	
Payments:		
Purchase of 2 shares Massachusetts Gas Companies, preferred	180.25	$244.83

LUCRETIA CROCKER FUND

Cash on hand January 1, 1915	$ 99.04	
Receipts:		
Interest —1/5 of $1,000 American Telephone & Telegraph Company 4 per cent. bond	8.00	
Dividend—18 shares Vermont & Massachusetts Railroad Company	108.00	
1 share West End Street Railway Company	3.50	
1 share American Telephone & Telegraph Company	8.00	
2½ shares General Electric Company	20.00	
	$246.54	
Payments:		
2 Scholarships	100.00	146.54

LIBRARY FUND

Cash on hand January 1, 1915	$174.16	
Receipts:		
Interest —4/5 of $1,000 American Telephone & Telegraph Company 4's	32.00	
¼ of $500 Western Telephone & & Telegraph Company, 5's	6.26	
Dividends—3 shares American Telephone & Telegraph Company	24.00	
1 share American Smelting & Refining Company, preferred	7.00	
2½ shares General Electric Company	20.00	
5 shares United Shoe Machinery Company, preferred	7.51	
2 shares Massachusetts Gas Companies, preferred	8.00	
	$278.93	
Payments:		
Purchase 2 shares Massachusetts Gas Companies, preferred	181.26	97.67
Cash on hand December 31, 1915		$489.04

INCOME AND EXPENSE FOR YEAR ENDED DECEMBER 31, 1915

	Expense	*Income*
Administration expense	$ 6,805.36	
BIOLOGICAL BULLETIN	2,594.69	$ 1,523.36
Boat department expense	6,629.63	
Carpenter department expense	983.09	
Chemical department expense	1,430.82	
Dormitories	1,489.49	1,691.04
Fish trap	1,159.21	667.21
Instruction	3,435.98	5,150.00
Philosophical lectures	100.00	
Library department expense	2,628.64	
Mess	15,753.01	16,165.48
Membership dues		1,000.00

Maintenance, buildings and grounds	5,220.23	
New laboratory expense	1,927.76	
Pumping station expense	464.70	
Research income		3,175.00
Sundry expense and income	2,165.48	3,255.25
Supply department	12,822.40	19,150.77
Interest on notes payable	150.00	
Total expense	$65,760.49	
Total income	51,778.11	$51,778.11
Excess of expense	$13,982.38	
Contribution by Mr. C. R. Crane	20,000.00	
Excess carried to balancing account	$ 6,017.62	

BALANCE-SHEET, DECEMBER 31, 1915

Assets		*Liabilities and Capital*	
Cash, bank	$ 4,212.16	Accounts payable	$ 1,394.02
Petty cash	200.00	Note payable	3,000.00
Accounts receivable	7,642.37	Trust funds	8,222.55
Investments	10,733.51	Balancing account	33,997.98
Investment cash	489.04		
Inventories			
Plant account			
Improvements, 1915	23,337.47		
	$46,614.55		$46,614.55

D. BLAKELY HOAR, ESQ., Treasurer,
Marine Biological Laboratory
Woods Hole, Mass.
161 Devonshire Street, Boston.

Dear Sir: We have audited the accounts of the Marine Biological Laboratory as kept at Woods Hole and of the trust funds and accounts as kept at your office, 161 Devonshire Street, for the year ended December 31, 1915.

We have checked the report of the Treasurer submitted above and find it correct and in accord with the books. The extent of the audit together with the supporting schedule is set forth in a detailed report under date of February 1, 1916.

Very respectfully,

HARVEY S. CHASE & CO.,
Certified Public Accountants

V. LIBRARIAN'S REPORT

AUGUST, 1915

Since the last annual report the reorganization of the library has been carried on by Miss Scott, who has brought each department up to a state of system and efficiency.

We can now make a more definite statement of the contents of the library, including a number of accessions not previously listed: Total number of accessions 10,046. Old and new accessions recorded since last report 6,746. Of these there have been added since 1914:

934 volumes loaned by the American Museum of Natural History,
466 volumes given by the Wisconsin Academy of Sciences,
200 " given by Mr. Crane,
720 " bound during year.

These volumes may be conveniently divided as follows:

Journals, for which most of our appropriation is expended and which form the bulk of the library, in bound sets	95
Serials and publications of academies, museums, laboratories, and societies (many of these are from exchanges which are steadily increasing)	188
Books:	
Zoology	321
Botany	134
Physiology	100
Hygiene and medicine	74
Chemistry	18
Evolution	82
Psychology	36
Philosophy	104
Miscellaneous	198
Total	1,067

We can buy very few books and are dependent upon gifts from authors and publishers, who recognize that this is a particularly useful place to have their works examined.

The separates number 4,000. Our reprint collection might easily be notably increased through the aid of authors and friends. Such duplicates are in great demand.

The following advances have been made. First, and very important, is the considerable number of missing parts which have been secured by Miss Scott's enterprise toward completing back volumes of sets.

These have been completed: *Nature*, *Wilson Bulletin*, *University of California Publications in Zoölogy*, *Colorado University Studies*, *Bergens Museum Publications.*

These have been added to but are still incomplete:

Annales d. Sciences Naturelles—Zoölogy,
Bulletin Museum of Comparative Zoölogy, Harvard,
Proceedings Boston Society of Natural History,
Proceedings American Philosophical Society,
American Naturalist,
American Journal of Science and Arts,
Journal Medical Research,
Field Columbian Museum Publications,
Bureau of Fisheries, Bulletin and Report,
Contributions U. S. National Herbarium,
Proceedings American Association for the Advancement of Science,
Proceedings Iowa Academy of Sciences,
Proceedings Indiana Academy of Sciences.

The following new sets have been added to the library:

Transactions American Microscopical Society,
Transactions Wisconsin Academy of Sciences,
Bulletin Torrey Botanical Club,
American Journal of Botany,
Proceedings Academy of Natural Sciences, Philadelphia,
Journal of Parasitology,
Illinois Biological Monographs,
Unpopular Review,
Annals Missouri Botanical Garden,
Indiana University Studies,
U. S. Bureau of Education, Report and Bulletin,
U. S. War Department, Bulletin,
Maine Agricultural Experiment Station, Report and Bulletin,
Museum of Brooklyn Institute of Arts and Sciences, Science Bulletin.

Seventy-six volumes of Hoppe-Seyler's *Zeitschrift für Physiologische Chemie* were purchased from Miss Koch for $200, and the remainder of the set, which is now in the 94th volume, has been secured.

Exchanges now number 29; six have been arranged this year: *Indian Museum; American Journal of Botany; New York Academy of Sciences; Academy of Natural Sciences, Philadelphia; Biologische Untersuchungen, Stockholm;* and *Zoologiska Bidrag*, Upsala. New subscriptions have been placed for the *Popular Science Monthly* and the *English Journal of Physiology.*

Here we must report a partial disposition of the Journal Fund which was begun in 1912. Since then, a number have subscribed to this fund which is to enable us to secure new files of journals. Drs. Mayer, Knower, Meigs, Rice and Just have subscribed from $5 to $10 a year for a period of five years. This has already furnished $55 and will still accumulate from new gifts.

In addition, last year Mr. Tashiro and a number of other investigators, interested in securing the *English Journal of Physiology*, subscribed amounts from 50 cents to $1 each, thus securing $7.50. This sum has now been increased to $12. For this, we must thank Drs. Tashiro, Heilbrunn, Gould, Wherry, Packard, Sturtevant, Bridges, Harvey, Metz, and Morgan, and Misses Hoge, Medes, Browne, and Dunn. Such coöperation is most encouraging. It makes possible a purchase long needed. We have subscribed to the *Journal* with this gift and have used the amount already accumulated for the Journal Fund to purchase a number of back volumes, hoping gradually complete to the set.

Further growth in this spirit of coöperation in building up the library is shown in the list of important gifts.

Various publishers have given a total of 16 books this year. Miss Fay has given several quite valuable books on mushrooms. Mr. Crane gave us two hundred volumes from his personal library, as well as two very interesting Russian curios. Dr. George T. Moore gave duplicates from his library, 28 bound volumes and 21 separates. Dr. Philipp Fischelis donated 53 valuable papers by Kowalewsky and other Russian authors. Dr. Conklin has given his book on "Heredity and Environment," Dr. Parker, his book on "Biology and Social Problems," and Dr. Abbott has ordered a copy of his book on "General Biology" sent to us. Dr. Calkins also presented his texbook on "Biology."

The Wistar Institute gave a set of the "Biological Lectures," Mrs. Gardiner donating Volume I. to complete our set. Dr. Ward gave a subscription to the *Journal of Parasitology*, and Dr. Bumpus to the current volume of the *American Naturalist* and the *Unpopular Review*. Dr. Gustav Retzius has promised to send a complete set of his works as soon as transportation is safer. Three hundred and twelve reprints have been presented to the library this year by various authors.

Through the coöperation of Dr. George Wagner, a large number of duplicates from the library of the Wisconsin Academy of Sciences are being transferred to this library; 466 volumes have been received and the number will probably reach 1,000.

The Prince of Monaco has sent us about 75 photographs of the buildings and equipment of the Institut Oceanographique, together with pamphlets telling about the foundation and progress of the institute. A number of other stations have sent various literature and photographs, and it is hoped to secure material of this kind from all biological stations in the world.

We wish to acknowledge the coöperation of Drs. Moore and Duggar in securing the *Bulletin of the Torrey Botanical Club*, and of Dr. Ivey Lewis in arranging and preparing for the binder a most confusing set of plates.

We are really in need of a number of journals that we do not have, and we should complete as soon as possible certain sets that have long been defective. The library needs an increased appropriation for handbooks, monographs, and general works like the Encyclopedia Britannica, the Century Dictionary, etc.

Above all, we must point out the great value of reprints and books sent here by individuals, and the importance of personal efforts in behalf of the library on the part of the biologists who use it. Such interest has already done much to build it up.

VI. THE DIRECTOR'S REPORT

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY:

Gentlemen: The season of 1915, the twenty-eighth session of the Marine Biological Laboratory, was marked by a farther advance in many of the statistics of the Laboratory as will appear from the appended lists (1) of the Staff, (2) of the Investigators and Students, (3) the Tabular View of Attendance, (4) the Subscribing Institutions, (5) of the Evening Lectures, (6) of the Members of the Corporation. The Treasurer's report and the Librarian's report also show a healthy condition of growth. We have much cause for congratulation, and reason for deep thankfulness to Mr. Crane without whose whole-hearted financial support and intelligent sympathy with our objects, our best efforts must have been comparatively ineffective.

The number of investigators in attendance was 137 as compared with 129 in 1914, 122 in 1913 and 93 in 1912; the number of students was 105 as compared with 89 in 1914, 69 in 1913 and 67 in 1912. The total attendance was 242 as compared with 218 in 1914, 191 in 1913 and 160 in 1912. These figures bear testimony to the growing appreciation of the facilities furnished by the Laboratory both in research and in instruction. The number of institutions represented by these workers was 79, which is almost the same as in the two preceding years; the increase was therefore due to larger attendance from certain institutions. Indeed no considerable increase in the number of institutions represented is to be expected, because practically all of the larger institutions of higher education in the East and Middle West are represented each year, and the variations from year to year are accounted for by fluctuations of a more or less fortuitous character among the smaller institutions represented.

The receipts from subscribing institutions and fees were $8,325 as compared with $7,300 in 1914, $6,160 in 1913 and $5,175 in 1912. The Supply Department has also shown an encouraging increase, having filled 282 more orders than in 1914 with total paid receipts of $16,932.00 as compared with $14,003.35 in 1914.

Three important additions to the property and equipment of the Laboratory were made during the year: (1) The so-called Bake House property adjoining the Laboratory property on the east was purchased; this piece has about 103 feet frontage on the Eel pond and on the main street by 100 feet in depth, and its title carries with it the private roadway previously controlled jointly by the Laboratory and the owners of this property. The old house on the property has been fitted up as a workshop for the carpenters' and plumbers' department, which was very badly needed. The frontage on the eel pond is especially valuable for future development. (2) The Laboratory also purchased the grounds and house known as the Ritter property, 78½ by 100 feet, adjacent to the Whitman house, and opposite the lecture hall. The house furnished much needed enlargement of our dormitory facilities. (3) The old "Homestead" hitherto used as the matron's and helpers' house, adjoining the mess, was torn down and replaced by a much larger, modern and attractive dwelling house with accommodations for 43 persons. As all of this space was not needed for the mess, part of it was used for a woman's dormitory. We owe these three splendid additions to the facilities of the Laboratory, as I need hardly say, to the continued generosity, and thoughtfulness in the matter of all Laboratory needs, of the President of the Board of Trustees, Mr. Crane. Other improvements during the year were as follows: The steamer *Cayadetta* was thoroughly overhauled and her deck raised 18 inches at a cost of over $2,000, so that she is a much stauncher and more seaworthy boat than ever before. The filling in of the Laboratory harbor frontage, including the Yacht Club, has been completed; this will be graded and planted in the spring and the building moved to the east end of the frontage away from the center of the new laboratory building. Very considerable additions were made to the kitchen and laundry of the mess, power machinery electrically operated being installed for all the major operations, such as dish-washing, washing and drying of laundry, freezing ice-cream, etc. The work has thus been greatly lightened and facilitated. The Laboratory also transferred a 40-foot strip at the extreme north and west end of our Eel pond frontage to the town of Falmouth for the purposes of a boat landing, which has now been built at a cost of about $1,500, equally divided between the town and Laboratory. This was in pursuance of an agreement entered into with the town at the time of the construction of the drawbridge over the Eel pond entrance. It is therefore now possible to use the Eel pond as a harbor, and to secure delivery of supplies by this back entrance to the Laboratory property.

With all of the enlargements and additions of the past few years the Laboratory is still badly crowded during the height of the season. This applies to a certain extent to the actual accommodations for workers in the laboratory buildings, but more particularly to the housing accommodations in the village. We are planning to meet the former condition by restricting numbers in the larger classes; applications for admission will be received up to May, and appointments then made in accordance with the number of working places; if places are still available after such assignments later applicants can be admitted. It is probable that by means of various adjustments we can continue to care for all qualified investigators.

The housing accommodations in the village, however, raise a more serious question. They are entirely inadequate, and there is a consequent tendency for the prices of rooms to advance, which results in discouraging attendance. Investigators who wish to carry on regular work at the Laboratory are unable to rent houses at reasonable rates for the accommodation of their families. Conditions have become increasingly discouraging in these respects for the past several years. Moreover, land is almost unavailable for those families who wish to solve the living problem by building. It is undoubted that these conditions will exercise an inhibiting influence on the work of the Laboratory in the future, and one of the most important problems before us is to devise some means of counteracting them.

As an organization we have laid the principle of coöperation at our foundation, and we have attempted to build it into every one of our activities. Our Board of Trustees is representative of the institutions of learning of the country; our corporation represents the workers in biology on the broadest lines we can secure; our staff of instructors is widely drawn from different institutions; our students and investigators come from all parts of the accessible territory, and some from abroad; we ask only that they have a fit preparation and exhibit the spirit of scholarship. We are not tied down to any one institution or small section of the country. In past years we have considered these principles worth struggling for, and we have more than once sacrificed security to freedom of action. Our recent years have been free and comfortable. We must not allow ourselves to forget that the principles for which we stand are never entirely won; and I appeal therefore to every member of the Board of Trustees and to all members of the corporation not to allow a sense of security to dull the edge of devotion, to keep the interests of the Laboratory at heart and to work for their support in all possible ways.

I. THE STAFF

1915

FRANK R. LILLIE, Director,
Professor of Embryology, and Chairman of the Department of Zoölogy, The University of Chicago.

GILMAN A. DREW, Assistant Director,
Marine Biological Laboratory.

ZOÖLOGY

I. INVESTIGATION

Gary N. Calkins	Professor of Protozoölogy, Columbia University.
E. G. Conklin	Professor of Zoölogy, Princeton University.
Gilman A. Drew	Assistant Director, Marine Biological Laboratory.
George Lefevre	Professor of Zoölogy, The University of Missouri.
Frank R. Lillie	Professor of Embryology, The University of Chicago.
C. E. McClung	Professor of Zoölogy, University of Pennsylvania.
T. H. Morgan	Professor of Experimental Zoölogy, Columbia University.
E. B. Wilson	Professor of Zoölogy, Columbia University.

II. INSTRUCTION

Caswell Grave	Associate Professor of Zoölogy, Johns Hopkins University.
W. C. Allee	Assistant Professor of Zoölogy, University of Oklahoma.

George A. Baitsell	Instructor in Biology, Yale University.
Raymond Binford	Professor of Biology, Earlham College.
E. J. Lund	Instructor in Protozoölogy, University of Pennsylvania.
T. S. Painter	Instructor in Biology, Yale University.

EMBRYOLOGY

I. INVESTIGATION (see Zoölogy)

II. INSTRUCTION

William E. Kellicott	Professor of Biology, Goucher College.
Robert A. Budington	Professor of Zoölogy, Oberlin College. (Absent in 1915.)
J. F. Abbott	Professor of Zoölogy, Washington University.
Charles Packard	Instructor in Zoölogy, Columbia University.
Charles C. Rogers	Professor of Zoölogy, Oberlin College.

PHYSIOLOGY

I. INVESTIGATION

Albert P. Mathews	Professor of Physiological Chemistry, The University of Chicago.
Ralph S. Lillie	Professor of Biology, Clark University.
Harold C. Bradley	Assistant Professor of Physiological Chemistry, University of Wisconsin.
Shiro Tashiro	Instructor in Physiological Chemistry, The University of Chicago.

II. INSTRUCTION

Ralph S. Lillie	Professor of Biology, Clark University.
Walter E. Garrey	Associate Professor of Physiology, Washington University Medical School.
Frank P. Knowlton	Professor of Physiology, Syracuse University.
Edward B. Meigs	Associate in Physiology, Wistar Institute of Anatomy and Biology.

PHILOSOPHICAL ASPECTS OF BIOLOGY AND ALLIED SCIENCES

LECTURES

Edward G. Spaulding	Professor of Philosophy, Princeton University.

BOTANY

George T. Moore	Director, Missouri Botanical Garden and Professor of Botany, Washington University.
B. M. Duggar	Physiologist, Missouri Botanical Garden and Professor of Plant Physiology, Washington University. (Absent in 1915.)
Ivey F. Lewis	Professor of Botany, University of Missouri.
R. H. Colley	Instructor in Botany, Dartmouth College.
A. R. Davis	Lackland Research Fellow, Missouri Botanical Garden.
W. H. Weston	Graduate Student, Harvard University.

LIBRARY

H. McE. Knower	Professor of Anatomy, University of Cincinnati, Librarian.
Mary E. Scott	Assistant Librarian.

Chemical Supplies

Oliver S. Strong	Instructor in Anatomy, College of Physicians and Surgeons, New York City, Chemist.

Supply Department

G. M. Gray	Curator.
John J. Veeder	Captain.
E. M. Lewis	Engineer.
A. W. Leathers	Collector.
A. M. Hilton	Collector.
F. G. Gustafson	Collector in Botany.
Edna E. Wells	Clerk.

F. M. MacNaught	Business Assistant.
Herbert A. Hilton	Superintendent of Buildings and Grounds.

2. INVESTIGATORS AND STUDENTS

1915

A. ZOÖLOGY

Independent Investigators

Abbott, James F., Professor of Zoölogy, Washington University.

Addison, William H. F., Assistant Professor of Normal Histology and Embryology, University of Pennsylvania.

Allee, Warder C., Professor of Biology, Lake Forest College.

Allen, Ezra, Professor of Biology, Philadelphia School of Pedagogy.

Baitsell, George A., Instructor in Biology, Yale University.

Beckwith, Cora J., Associate Professor of Zoölogy, Vassar College.

Binford, Raymond, Professor of Zoölogy, Earlham College, Richmond.

Boring, Alice M., Associate Professor of Zoölogy, University of Maine.

Browne, Ethel N., 510 Park Ave., Baltimore, Md.

Calkins, Gary N., Professor of Protozoölogy, Columbia University.

Casteel, Dana B., Associate Professor of Zoölogy, University of Texas.

Chidester, Floyd E., Associate Professor of Zoölogy, Rutgers College.

Child, C. M., Associate Professor of Zoölogy, University of Chicago.

Clapp, Cornelia M., Professor of Zoölogy, Mt. Holyoke College.

Clark, Eleanor L., Columbia, Mo.

Clark, Eliot R., Professor of Anatomy, University of Missouri.

Conklin, Edwin G., Professor of Biology, Princeton University.

Copeland, Manton, Professor of Biology, Bowdoin College.

Cowdry, E. V., Associate in Anatomy, Johns Hopkins Medical School.

Cowdry, N. H., Johns Hopkins Medical School, Baltimore, Md.

Crampton, H. E., Professor of Zoölogy, Barnard College, Columbia Univ.

Danchakoff, Wera, Woods Hole, Mass.

Dexter, John S., Professor of Biology, University of Saskatchewan.

Donaldson, H. H., Wistar Institute of Anatomy and Biology.

Drew, Gilman A., Assistant Director, Marine Biological Laboratory.

Dunn, Elizabeth H., Woods Hole, Mass.

Gee, Wilson, Professor of Biology, Emory University, Oxford, Ga.

Goldfarb, A. J., Professor of Biology, College of the City of New York.

Goldschmidt, Richard, Member of Kaiser Wilhelm Institut Für Biologie.

Grave, Caswell, Associate Professor of Zoölogy, Johns Hopkins University.

Gregory, Emily R., Professor of Biology, University of Akron.

Gregory, Louise H., Instructor in Zoölogy, Barnard College.

Gross, Alfred O., Bowdoin College, Brunswick, Maine.

Harman, Mary T., Assistant Professor of Zoölogy, Kansas State Agricultural College.

Harvey, Basil C. H., Associate Professor of Anatomy, University of Chicago.

Heilbrunn, Lewis V., Associate in Embryology, University of Chicago.

Hegner, Robert W., Assistant Professor of Zoölogy, University of Michigan.

Hogue, Mary J., Instructor in Zoölogy, Wellesley College.

Jacobs, Merkel H., Assistant Professor of Zoölogy, University of Pennsylvania.

KELLICOTT, WILLIAM E., Professor of Biology, Goucher College.
KNOWER, HENRY MCE., Professor of Anatomy, University of Cincinnati.
LANE, HENRY H., Professor of Zoölogy, State University of Oklahoma.
LEFEVRE, GEORGE, Professor of Zoölogy, University of Missouri.
LEWIS, MARGARET R., Carnegie Institution.
LEWIS, WARREN H., Professor of Physiological Anatomy, Johns Hopkins Medical School.
LILLIE, FRANK R., Professor of Embryology, University of Chicago.
LUND, ELMER J., Assistant Professor of Zoölogy, University of Minnesota.
MALONE, EDWARD F., Associate Professor of Anatomy, University of Cincinnati.
MACKLIN, CHARLES C., Johns Hopkins Medical School, Baltimore, Md.
MCCLUNG, CLARENCE E., Director of Zoölogical Laboratory, University of Pennsylvania.
MORGAN, T. H., Professor of Experimental Zoölogy, Columbia University.
MORGAN, ANNA H., Associate Professor of Zoölogy, Mt. Holyoke College.
MORRIS, MARGARET, Fellow in Zoölogy, Yale University.
PACKARD, CHARLES, Instructor in Zoölogy, Columbia University.
PAINTER, THEOPHILUS S., Instructor in Biology, Yale University.
PAPPENHEIMER, ALWIN M., Assistant Professor of Pathology, Columbia Univ.
PATTERSON, JOHN T., Professor of Zoölogy, University of Texas.
RICHARDS, A., Instructor in Zoölogy, University of Texas.
ROBERTSON, W. REES B., Assistant Professor of Zoölogy, University of Kansas.
SPAETH, REYNOLD A., Instructor in Biology, Yale University.
SPAULDING, E. G., Professor of Philosophy, Princeton University.
STOCKARD, CHARLES R., Professor of Anatomy, Cornell Medical College.
STREETER, GEORGE L., Research Associate, Carnegie Institution of Washington.
STRONG, OLIVER S., Instructor in Anatomy, Columbia University.
STURTEVANT, ALFRED H., Columbia University, New York City.
TENNENT, DAVID H., Professor of Biology, Bryn Mawr College.
WELCH, PAUL S., Assistant Professor of Entomology, Kansas State Agricultural College.
WOODWARD, ALVALYN E., Fellow in Zoölogy, University of Michigan.
ZELENY, CHARLES, Associate Professor of Zoölogy, University of Illinois.

Beginning Investigators

ADKINS, W. S., Graduate Student, Columbia University.
ALTENBURG, EDGAR, Assistant in Botany, Columbia University.
BINKLEY, LELIA T., University of Texas.
BRIDGES, CALVIN B., Columbia University.
COHN, EDWIN J., University of Chicago.
COLLETT, MARY E., Instructor in Biology, Carnegie Institution of Technology.
FISH, J. Burton, Teacher of Biology, Boys' High School, Brooklyn.
FREIBERG, HENRY B., University of Cincinnati.
GLOBUS, JOSEPH H., Assistant in Anatomy, Cornell Medical College.
GOLDMAN, AGNES, Cornell University.
GOODRICH, H. B., Union College, Schnectady.
GOULD, HARLEY N., Fellow in Biology, Princeton University.
HANCE, ROBERT T., Fellow in Zoölogy, University of Pennsylvania.
HASTINGS, GEORGE T., Teacher of Biology, DeWitt Clinton High School, Yonkers, N. Y.

Hayden, Margaret A., Instructor in Biology, Carnegie Institute of Technology.
Holt, Caroline M., Graduate Student, University of Pennsylvania.
Hickernell, Louis M., Instructor in Zoölogy, Syracuse University.
Hoy, William E., Jr., Research Student, Princeton University.
Johnson, Sydney E., Fellow in Zoölogy, Northwestern University.
Lynch, Clara J., Instructor, Smith College.
Marks, Bernice, 4 East 94th St., New York City.
Medes, Grace, Graduate Student, Bryn Mawr College.
Minoura, Tadachika, Zoölogy Department, University of Chicago.
Moore, Carl R., University of Chicago.
Osterud, Hjalmar L., Instructor in Zoölogy, University of Washington
Phillips, Ruth L., Associate Professor of Biology, Western College.
Reagan, Franklin T., Fellow in Biology, Princeton University.
Root, Francis M., Johns Hopkins University.
Safir, Shelley R., Teacher in New York High School, New York City.
Vanneman, Aimée S., Technician, University of Texas.
Ware, Clara C., Graduate Student, Columbia University.
Weinstein, Alexander, Columbia University.
Wheat, Frank M., Columbia University.
Whiting, Phineas W., University of Pennsylvania.
White, Edith G., Columbia University.
Wilkins, Lawson, Johns Hopkins University.

B. PHYSIOLOGY

Independent Investigators

Bogacki, Kamil J., Physician, Western Reserve University.
Chambers, Robert, Jr., Assistant Professor of Histology and Comparative Anatomy, University of Cincinnati.
Edwards, Dayton J., Instructor in Physiology, College of the City of New York.
Garrey, Walter E., Associate Professor of Physiology, Washington University.
Hyde, Ida H., Professor of Physiology, University of Kansas.
Just, Ernest E., Professor of Physiology, Howard University.
Kingsbury, Francis B., Instructor in Physiological Chemistry, University of Minnesota.
Knowlton, Frank P., Professor of Physiology, Syracuse University.
Lillie, Ralph S., Professor of Biology, Clark University.
Loeb, Jacques, Head of Department of Experimental Biology, Rockefeller Institute for Medical Research.
Mathews, Albert P., Professor of Physiological Chemistry, University of Chicago.
Meigs, Edward B., 1722 H. Street, N. W., Washington, D. C.
Moore, Arthur R., Associate Professor of Physiology, Bryn Mawr College.
Rogers, Charles G., Professor of Comparative Physiology, Oberlin College.
Scott, Ernest L., Associate in Physiology, Columbia University.
Tashiro, Shiro, Instructor in Physiological Chemistry, University of Chicago.
Waller, John C., Graduate Student, University of Chicago.
Warren, Howard C., Professor of Psychology, Princeton University.
Wasteneys, Hardolph, Associate in Experimental Biology, Rockefeller Institute for Medical Research.
Werber, Ernest L. Sessel Research Fellow, Yale University.

Beginning Investigators

Atwood, W. G., Dartmouth College.
Chamberlain, Mary M., Bryn Mawr College.
Levy, Augustus, University of Chicago.
Cattell, McKeen, Harvard Medical School.

C. BOTANY

Independent Investigators

Blakeslee, A. F., Professor of Botany and Genetics, Connecticut Agricultural College.
Colley, R. H., Instructor in Botany, Dartmouth College.
Hibbard, R. Percival, Plant Physiologist, Michigan Agricultural College.
Lewis, Ivey F., Professor of Botany, University of Missouri.
Moore, George T., Director, Missouri Botanical Garden, St. Louis.
Weston, William H., Jr., Instructor in Botany, Harvard University.

Beginning Investigators

Cobb, Ruth, Falls Church, Virginia.
Smith, Pearl M., Assistant in Botany, University of Wisconsin.

STUDENTS

1915

1. ZOÖLOGY

Adams, A. Elizabeth, Instructor in Zoölogy, Mount Holyoke College.
Andrus, Edwin C., Student, Oberlin College, Oberlin, Ohio.
Andrus, William D. W., Student, Oberlin College, Oberlin, Ohio.
Bain, Therese S., Vassar College, Poughkeepsie, N. Y.
Baldwin, Imogen, Student, Mt. Holyoke College, So. Hadley, Mass.
Bell, Charles E., Student, Ursinus College.
Blanchard, Nellie P., Head of Biological Dept., Hood College.
Cattell, Owen, Garrison-on-Hudson.
Cattell, Psyche, Student, Sargent, Cambridge, Mass.
Charlton, Harry H., Assistant in Zoölogy, Yale University.
Cocks, Edmund, Graduate Student, Columbia University, New York City.
Cobb, Margaret C., Barnard College, New York City, N. Y.
Cowdery, Lawrence T., Student, Oberlin College, Oberlin, Ohio.
Cross, Howard B., Instructor, Oklahoma University, Norman, Okla.
Dibell, Mabel E., Instructor of Biology, Western College.
Ehrenfeld, Frederick, Assistant Professor of Geology, University of Pennsylvania.
Gilbert, Marion, Student, Oberlin College, Oberlin, Ohio.
Greisheimer, Esther M., Clark University, Worcester, Mass.
Harvey, Helen F., Oberlin College, Oberlin, Ohio.
Harris, Coleman J., Lewisburg, Pa.

Hayden, Ruth, Goucher College, Baltimore, Md.
Hea, Emily M., Teacher in High School, Morristown, N. J.
Heeman, Harriet M., Student, Oberlin College, Oberlin, Ohio.
Howe, Marion G., Mt. Holyoke College, So. Hadley, Mass.
Hughes, Dorothea M., Simmons College, Boston, Mass.
Hulst, Myra M. Vassar College, Poughkeepsie, N. Y.
Irvine, Helen, Student, Mt. Holyoke College, So. Hadley, Mass.
Josephs, H. W., Assistant in Chemistry, Harvard University, Cambridge.
Kostir, Wencel J., Instructor, Ohio State University, Columbus, Ohio.
Ludwig, Albert P., Student, Oberlin College, Oberlin, Ohio.
Marinus, Carleton J., Syracuse University, Syracuse, N. Y.
Mahr, Ernst F., Jr., Syracuse University, Syracuse, N. Y.
McGrath, Jule G., Laboratory Assistant, Hunter College, New York City.
Moses, Gertrude, 2002 Bolton St., Baltimore, Md.
Oudesluys, Hortense, Teacher, Western High School, Baltimore, Md.
Pennypacker, Frances W., Sweet Briar College.
Ripple, Clarence V., Student, University of Pennsylvania, Philadelphia.
Rogers, Mildred, Student, Goucher College, Baltimore, Md.
Runyon, Paul M., Student, Princeton University, Princeton, N. J.
Sanford, Eldon W., Graduate Student, Yale University, New Haven, Conn.
Sheldon, Paul B., Student, Oberlin College, Oberlin, Ohio.
Smith, Sue F., Carnegie Institute of Technology.
Smith, Christianna, Assistant in Zoölogy Laboratory, Mt. Holyoke College, So. Hadley, Mass.
Smith, Inez C., Student, Mt. Holyoke College, So. Hadley, Mass.
Spence, Margaret, Instructor La Crosse State Normal School, La Crosse.
Wilder, Katherine, Science Assistant, The Newton High School.
Wright, Helen G., Student, Mt. Holyoke College, So. Hadley, Mass.

2. EMBRYOLOGY

Ashman, Richard, Rutgers College, New Brunswick, N. J.
Allard, Ann D., Teacher, So. Boston, Mass.
Blanchard, Frank H., Instructor, Tufts College, Mass.
Blau, Julia E., Princeton, N. J.
Cahn, Alvin R., Assistant in Zoölogy, University of Wisconsin, Madison, Wis.
Carroll, Mitchel, 617 So. 16th St., Philadelphia, Pa.
Closson, James H., Student, Princeton University, Princeton, N. J.
Cobb, Dorothy, Student, Radcliffe College, Cambridge, Mass.
Cutter, Elizabeth, Assistant in Zoölogy, Vassar College.
Farnum, Alice R., Student, Smith College, Northampton, Mass.
Fish, Gordon T., Assistant, Yale University, New Haven, Conn.
Hamlin, Howard E., Student, Harvard University, Cambridge, Mass.
Henry, Edna M., Student, Barnard College, New York City, N. Y.
Herrick, Joseph C., Professor of Biology, St. Joseph's Seminary, Yonkers, N. Y.
Hufford, Clarence E., Student, Oberlin College, Oberlin, Ohio.
Hurlin, Ralph G., Instructor, Clark College.
Jamieson, Janet P., Student, University of Pennsylvania.
Kakiuchi, Samuro, Yale University, New Haven, Conn.
Lewis, Elsie M., Student, Oberlin College, Oberlin, Ohio.

Macy, Cora F., Student, Syracuse University, Syracuse, N. Y.
Mallard, Agnes K., Teacher, 1658 Columbia Road, So. Boston, Mass.
Myers, Mae L., Associate Professor of Anatomy, Woman's Medical College of Pennsylvania.
Millikan, Frances, Student, Smith College, Northampton, Mass.
Montgomery, Priscilla B., 105 So. 41 St., Philadelphia, Pa.
Parmenter, Charles L., Assistant Professor, University of Wisconsin.
Patten, Mary W., Goucher College, Baltimore, Md.
Richards, Lyman G., 259 Prospect St., Fall River, Mass.
Richards, George L., 124 Franklin St., Fall River, Mass.
Sherwood, Helen L., Student, Vassar College, Poughkeepsie, N. Y.
Stocking, Ruth J., Professor of Biology, Agnes Scott College, Decatur.
Stocking, Besse E., Student, Goucher College, Baltimore, Md.
Straus, Aubrey H., Associate Professor of Bacteriology, Medical College of Virginia, Richmond, Va.
Thomas, Anna M., Student, Carnegie Institute of Technology.
Webster, Leslie T., Amherst College, Amherst, Mass.
Williams, G. Huntington, Graduate Student, Johns Hopkins Medical School.
Williams, James W., Lincoln St., New Haven, Conn.
Winslow, Mina L., Graduate Student, University of Michigan.

3. PHYSIOLOGY

Adler, Francis H., University of Pennsylvania, Philadelphia, Pa.
Bensing, LeRue P., Research Assistant, Research Commission of Natural Dental Association, Cleveland, Ohio.
Bodine, Joseph H., University of Pennsylvania, Philadelphia, Pa.
Fenn, Wallace O., Harvard University, Cambridge, Mass.
Eggstein, A. A., Instructor in Pathology, Vanderbilt College.
Holt, Emmett, 14 West 55th St., New York City, N. Y.
Hughes, Walter S., Massachusetts Institute of Technology.
Loeb, Robert F., Student, University of Chicago, Chicago, Ill.
Lundgren, C. Albert, Assistant in Biology, Clark University.
Metcalf, John T., Instructor in Psychology, Princeton University.
Minnich, Dwight E., Harvard University, Cambridge, Mass.
Price, Weston A., Chairman, The Research Commission of the National Dental Association, Cleveland, Ohio.
Reeves, Prentice, Assistant in Experimental Psychology, Princeton Univ.
Schneider, Peter A., Instructor in Zoölogy, University of Vermont.
Thompson, Martha, Teacher of Biology, Bay Ridge High School, New York City, N. Y.

4. BOTANY

McConnell, Louise J., 341 Rector St., Perth Amboy, N. J.
Mong, Grace E., Student, Oberlin College, Oberlin, Ohio.
Oak, Dorothy, Student, Barnard College, New York City, N. Y.
Severy, J. Warren, Student, Oberlin College, Oberlin, Ohio.
Yasui, Kono, Student, Radcliffe College, Cambridge, Mass.
Young, Anna R., Student, Smith College, Northampton, Mass.

3. TABULAR VIEW OF ATTENDANCE

	1911	1912	1913	1914	1915
Investigators—Total	82	93	122	129	137
Independent:					
Zoölogy	42	44	58	62	69
Physiology	18	14	17	22	20
Botany	8	10	11	10	6
Under Instruction:					
Zoölogy	12	21	21	31	36
Physiology	2	2	7	1	4
Botany		2	7	3	2
Students—Total	65	67	69	89	105
Zoölogy	26	24	33	43	47
Embryology	20	15	22	21	37
Physiology	6	11	8	10	15
Botany	13	17	7	15	6
Total Attendance	147	160	191	218	242
Institutions Represented—					
Total		57	80	77	79
By investigators	37	43	50	51	59
By students	31	36	41	47	42
Schools and Academies Represented.					
By investigators	3	2	3	1	3
By students	9	1	6	5	9

4. SUBSCRIBING INSTITUTIONS—1915

Amherst College
Barnard College
Bowdoin College
Bryn Mawr College
Carnegie Institute of Technology
Carnegie Institution, Washington
Clark University
Columbia University
Dartmouth College
Else Seringhaus Scholarship, Hunter College, New York City
Goucher College

Harvard University
Johns Hopkins University
Kansas State Agricultural College
Lucretia Crocker Scholarships
Mount Holyoke College
Northwestern University
Oberlin College
Princeton University, Dept. of Biology
Princeton University, Dept. of Psychology
Radcliffe College
Rockefeller Institute for Medical Research
Rutgers College
Smith College
Syracuse University
University of Chicago
University of Illinois
University of Kansas
University of Michigan
University of Pennsylvania
University of Texas
University of Wisconsin
Vassar College
Wellesley College
Western College
Wistar Institute
Yale University

5. EVENING LECTURES, 1915

Friday, July 2,
Prof. C. R. Stockard.. "Experimental Production of Racial Degeneracy by Alcohol Poisoning."

Tuesday, July 6,
Prof. C. H. Parker.... "The Seals of the Pribiloff Islands."

Friday, July 9,
Prof. G. L. Streeter.. "Some Experimental Studies on the Development of the Membranous Labyrinth in the Tadpole."

Tuesday, July 13,
Prof. E. G. Conklin... "Effects of Centrifugal Force on the Structure and Development of the Egg."

Friday, July 16,
Prof. R. S. Lillie . . . . . " The Nature of Intelligent and Purposive Action from a Physiological Point of View."
Monday, July 19,
Prof. Simon Flexner . . " The Control of Infection as Affected by Variation Among Parasitic Microörganisms."
Saturday, July 24,
Prof. G. N. Calkins . . . " Protozoa and the Cancer Problem."
Friday, July 30,
Prof. George Shull . . . " Inheritance of Sex in *Lychnis*."
Tuesday, Aug. 3,
Prof. C. B. Davenport " Heredity of Criminality."
Friday, Aug. 6.
Dr. Martin Edwards . . " The Story of Bubonic Plague."
Tuesday, Aug. 10,
Dr. Alfred G. Mayer . " The Rôle of Adsorption in Nerve Conduction."

6. MEMBERS OF THE CORPORATION

1. Life Members

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Md.
Billings, Mr. R. C., 66 Franklin St., Boston, Mass.
Carey, Mr. Arthur Astor, Fayerweather St., Boston, Mass.
Clarke, Prof. S. F., Williams College, Williamstown, Mass.
Conklin, Prof. Edwin G., Princeton University, Princeton, N. J.
Crane, Mr. C. R., Woods Hole, Mass.
Davis, Major Henry M., Syracuse, N. Y.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Mass.
Farlow, Prof. W. G., Harvard University, Cambridge, Mass.
Fay, Miss S. B., 88 Mt. Vernon St., Boston, Mass.
Folsom, Miss Amy, 88 Marlboro St., Boston, Mass.
Foot, Miss Katherine, 80 Madison Ave., New York City, N. Y.
Gardiner, Mrs. E. G., Woods Hole, Mass.
Gardiner, Miss Eugenia, 15 W. Cedar St., Boston, Mass.
Hannaman, Mr. Charles E., 103 1st St., Troy, N. Y.

Harrison, Ex-Provost C. C., University of Pennsylvania, Philadelphia, Pa.
Jackson, Miss M. C., 88 Marlboro St., Boston, Mass.
Jackson, Mr. Chas. C., 24 Congress St., Boston, Mass.
Kennedy, Mr. Geo. G., 284 Warren St., Roxbury, Mass.
Kidder, Mr. C. G., 27 William St., New York City, N. Y.
Kidder, Mr. Nathaniel T., Milton, Mass.
King, Mr. Chas. A.
Lee, Mrs. Frederic S., 279 Madison Ave., New York City, N. Y.
Lowell, Mr. A. Lawrence, 171 Marlboro St., Boston, Mass.
Marrs, Mrs. Laura Norcross, 9 Commonwealth Ave., Boston, Mass.
Mason, Mr. E. F., 1 Walnut St., Boston, Mass.
Mason, Miss Ida M., 1 Walnut St. Boston, Mass.
Means, Mr. James Howard, 196 Beacon St., Boston, Mass.
Merriman, Mrs. Daniel, Worcester, Mass.
Minns, Miss Susan, 14 Louisburg Square, Boston, Mass.
Minns, Mr. Thomas, 14 Louisburg Square, Boston, Mass.
Mixter, Miss M. C., 241 Marlboro St., Boston, Mass.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Sts., New York City, N. Y.
Morgan, Prof. T. H., Columbia University, New York City, N. Y.
Morgan, Mrs. T. H., New York City, N. Y.
Noyes, Miss Eva J., 28 South Willow St., Montclair, N. J.
Nunn, Mr. Lucian L. Telluride, Colo.
Osborn, Prof. Henry F., American Museum of Natural History, New York.
Phillips, Dr. John C., Windy Knob, Newham, Mass.
Phillips, Mrs. John C., Windy Knob, Newham, Mass.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pa.
Pulsifer, Mr. W. H., Newton Center, Mass.
Rogers, Miss A. P., 5 Joy St., Boston, Mass.
Sears, Dr. Henry F., 420 Beacon St., Boston, Mass.
Shedd, Mr. E. A.,
Smith, Mrs. C. C., 286 Marlboro St., Boston, Mass.
Strobell, Miss E. C., 80 Madison Ave., New York City, N. Y.
Thorndike, Dr. Edward L., Teachers College, Columbia University, New York City, N. Y.

Trelease, Prof. William, University of Illinois, Champaign, Ill.
Ware, Miss Mary L., 41 Brimmer St., Boston, Mass.
Warren, Mrs. S. D., 67 Mt. Vernon St., Boston, Mass.
Whitney, Mr. Henry M., Brookline, Mass.
Willcox, Miss Mary A., Wellesley College, Wellesley, Mass.
Wilmarth, Mrs. H. D., Elliott St., Jamaica Plain, Mass.
Williams, Mrs. Anna P., 505 Beacon St., Boston, Mass.
Wilson, Dr. E. B., Columbia University, New York City, N. Y.
Wilson, Prof. W. P., Philadelphia Museum, Philadelphia, Pa.

2. Members, January, 1916

Abbott, Prof. J. F., Washington University, St. Louis, Mo.
Abbott, Miss Margaret B., The Bennett School, Milbrook, N. Y.
Addison, Dr. W. H. F., University of Pennsylvania, Medical School, Philadelphia, Pa.
Adkins, Mr. W. S., Texas Christian University, Fort Worth, Texas.
Allee, Dr. W. C., Lake Forest College, Lake Forest, Ill.
Allen, Prof. Ezra, 125 Thompson Ave., Ardmore, Pa.
Allyn, Miss Harriet M., Hackett Medical College, Canton, China.
Alsburg, Dr. C. S., U. S. Dept. of Agriculture, Washington, D. C.
Baitsell, Dr. George A., Sheffield Scientific School, Yale University, New Haven, Conn.
Baker, Dr. E. H., 154 W. Randolph St., Chicago, Ill.
Bancroft, Dr. F. W., Aloha Farm, Concord, California.
Bardeen, Prof. C. R., University of Wisconsin, Madison, Wis.
Beckwith, Miss Cora J., Vassar College, Poughkeepsie, N. Y.
Behre, Miss Elinor H., University of Chicago, Chicago, Ill.
Beyer, Dr. H. G., Stoneleigh Court, Washington, D. C.
Bigelow, Prof. M. A., Teachers College, Columbia University, New York City, N. Y.
Bigelow, Prof. R. P., Mass. Institute of Technology, Boston, Mass.
Binford, Dr. Raymond, Earlham College, Richmond, Ind.

Binkley, Miss Lelia T., University of Texas, Austin, Texas.
Blakeslee, Prof. A. F., Carnegie Station, Cold Spring Harbor, Long Island.
Boring, Miss Alice M., University of Maine, Orono, Maine.
Box, Miss Cora May, University of Cincinnati, Cincinnati, Ohio.
Bradley, Dr. Harold C., University of Wisconsin, Madison, Wis.
Browne, Miss Ethel N., Cornell Medical School, New York City, N. Y.
Budington, Prof. R. A., Oberlin College, Oberlin, Ohio.
Bumpus, Dr. H. C., Tufts College, Mass.
Byrnes, Dr. Esther F., 193 Jefferson Ave., Brooklyn, N. Y.
Buckingham, Miss Edith N., 342 Marlboro St., Boston, Mass.
Calkins, Prof. Gary N., Columbia University, New York City, N. Y.
Calvert, Prof. Philip P., Univ. of Pennsylvania, Philadelphia, Pa.
Carlson, Prof. A. J., University of Chicago, Chicago, Ill.
Carver, Mr. Gail L., 307 Adams St., Macon, Georgia.
Cary, Dr. L. R., Princeton University, Princeton, N. J.
Casteel, Dr. D. B., University of Texas, Austin, Texas.
Cattell, Prof. J. McKeen, Garrison-on-Hudson, N. Y.
Cattell, Mr. McKeen, Harvard Medical School, Boston, Mass.
Chambers, Dr. Robert, Jr., Cornell Medical College, New York City, N. Y.
Chester, Prof. Webster, Colby College, Waterville, Maine.
Chidester, Dr. F. E., Rutgers College, New Brunswick, N. J.
Child, Prof. C. M., University of Chicago, Chicago, Ill.
Clapp, Prof. Cornelia M., Mount Holyoke College, South Hadley, Mass.
Clark, Dr. E. R., University of Missouri, Columbia, Mo.
Coe, Prof. W. R., Yale University, New Haven, Conn.
Colley, Dr. R. H., Dartmouth College, Hanover, N. H.
Colton, Dr. H. S., Ardmore, Pa.
Coolidge, Mr. C. A., Ames Bldg., Boston, Mass.
Copeland, Dr. Manton, Bowdoin College, Brunswick, Maine.
Cowdry, Dr. E. V., Johns Hopkins Medical School, Baltimore, Md.

CRAMPTON, PROF. H. E., Barnard College, Columbia University, New York City, N. Y.
CRANE, MRS. C. R., Woods Hole, Mass.
CURTIS, PROF. W. C., University of Missouri, Columbia, Mo.
DANCHAKOFF, MME. VERA, 451 57th St., New York City, N. Y.
DAVIS, MR. DONALD W., DePauw University, Greencastle, Ind.
DERICK, PROF. CARRIE M., McGill University, Montreal, Canada.
DEXTER, DR. J. S., University of Saskatchewan, Saskatoon, Saskatchewan.
DODDS, PROF. G. S., University of Missouri, Columbia, Mo.
DONALDSON, PROF. H. H., Wistar Institute of Anat. and Biol., Philadelphia, Pa.
DORRANCE, MISS ANN, Dorranceton, Pa.
DORRANCE, MISS FRANCES, Dorranceton, Pa.
DREW, PROF. GILMAN A., Marine Biological Laboratory, Woods Hole, Mass.
DUGGAR, PROF. B. M., Missouri Botanical Garden, St. Louis, Mo.
DUNGAY, DR. NEIL S., Carleton College, Northfield, Minn.
DUNN, DR. ELIZABETH, Woods Hole, Mass.
EATON, PROF. E. H., Hobart College, Geneva, N. Y.
EDWARDS, DR. D. J., College of the City of New York, New York City, N. Y.
EIGENMANN, PROF. C. H., University of Indiana, Bloomington, Ind.
EWALD, DR. W. F., Kaiserin Augusta Str. 78, Berlin W 10, Germany.
FARNUM, MISS LOUISE W., 43 Hillhouse Ave., New Haven, Conn.
FERGUSON, PROF. J. S., Cornell Univ. Medical School, New York City, N. Y.
FIELD, MISS HAZEL E., Randolph-Macon Woman's College, College Park, Va.
FIELD, PROF. IRVING, Auburn, Mass.
FISH, MR. J. BURTON, Boys' High School, Brooklyn, N. Y.
GAGE, PROF. S. H., Cornell University, Ithaca, N. Y.
GARREY, PROF. W. E., Washington University Medical School, St. Louis, Mo.
GEE, PROF. WILSON, Emory University, Oxford, Ga.

GIES, PROF. W. J., Columbia Univ., Dept. Physiological Chemistry, New York City, N. Y.

GLASER, PROF. O. C., University of Michigan, Ann Arbor, Mich.

GLASER, DR. R. W., Bussey Institution, Forest Hills, Mass.

GOLDFARB, PROF. A. J., College of the City of New York, New York City, N. Y.

GOODRICH, MR. H. B., Union College, Schenectady, New York.

GRAVE, DR. CASWELL, Johns Hopkins University, Baltimore, Md.

GREGORY, DR. LOUISE H., Barnard College, Columbia University, New York City, N. Y.

GREGORY, DR. EMILY R., 501 South 42nd St., Philadelphia, Pa.

GREENMAN, DR. M. J., Wistar Institute of Anat. and Biol., Philadelphia, Pa.

GROSS, MISS BEATRIX H., 457 Convent Ave., New York City, N. Y.

GUNTHER, MISS MAUD C., Eastern High School, Washington, D. C.

HAHN, DR. C. W., 567 West 186th St., New York City, N. Y.

HANCE, MR. ROBERT T., University of Pennsylvania, Philadelphia, Pa.

HARGITT, DR. C. W., Syracuse University, Syracuse, N. Y.

HARMAN, DR. MARY T., Kansas State Agricultural College, Manhattan, Kans.

HARPER, PROF. R. A., Columbia University, New York City, N. Y.

HARRISON, MR. A. C., 660 Drexel Bldg., 5th and Chestnut Sts., Philadelphia, Pa.

HARRISON, PROF. ROSS G., Yale University, New Haven, Conn.

HARVEY, PROF. B. C. H., University of Chicago, Chicago, Ill.

HARVEY, DR. E. N., Princeton University, Princeton, N. J.

HAUGHWONT, MR. F. G., College of Medicine and Surgery, Calle Herran, Manila, Philippine Islands.

HAYDEN, MISS MARGARET A., Carnegie Inst. of Technology, Pittsburgh, Pa.

HAYES, PROF. S. P., Mount Holyoke College, South Hadley, Mass.

HEATH, PROF. HAROLD, Stanford University, San Francisco, Cal.

HEGNER, PROF. R. W., University of Michigan, Ann Arbor, Mich.

HEILBRUNN, Dr. L. V., University of Chicago, Chicago, Ill.
HOAR, MR. D. BLAKELY, 161 Devonshire St., Boston, Mass.
HOGUE, DR. MARY J., Wellesley College, Wellesley, Mass.
HOGE, MISS MILDRED A., Univ. of Indiana, Arbutus Apts., Bloomington, Ind.
HOLMES, PROF. S. J., University of California, Berkeley, Cal.
ISAACS, MR. RAPHAEL, University of Cincinnati, Cincinnati, Ohio.
ISELEY, PROF. F. B., Central College, Fayette, Mo.
JACKSON, PROF. C. M., University of Minnesota, Minneapolis, Minn.
JACOBS, MR. MURKEL H., University of Pennsylvania, Philadelphia, Pa.
JENNINGS, PROF. H. S., Johns Hopkins University, Baltimore, Md.
JENNER, PROF. E. A., Simpson College, Indianola, Iowa.
JEWETT, PROF. J. R., Harvard University, Cambridge, Mass.
JONES, PROF. LYNDS, Oberlin College, Oberlin, Ohio.
JORDAN, PROF. H. E., University of Virginia, Charlottesville, Va.
JUST, PROF. E. E., Howard University, Washington, D. C.
KANDA, DR. SAKYO, University of Minnesota, Minneapolis, Minn.
KELLICOTT, PROF. W. E., Goucher College, Baltimore, Md.
KELLY, MR. J. P., 2163 Gleason Ave., Unionport, N. Y.
KENNEDY, DR. HARRIS, 286 Warren St., Roxbury, Mass.
KING, DR. HELEN DEAN, Wistar Institute, Philadelphia, Pa.
KINGSBURY, PROF. B. F., Cornell University, Ithaca, N. Y.
KINGSLEY, PROF. J. S., University of Illinois, Urbana, Ill.
KIRKHAM, DR. W. B., Yale University, New Haven, Conn.
KITE, Dr. G. L., Henry Phipps Institute, Philadelphia, Pa.
KNIGHT, MISS MARIAN V., 36 Bedford Terrace, Northampton, Mass.
KNOWER, PROF. H. McE., University of Cincinnati, Cincinnati, Ohio.
KNOWLTON, PROF. F. P., Syracuse University, Syracuse, N. Y.
KNUDSON, PROF. LEWIS, Cornell University, Ithaca, N. Y.
KRIBS, DR. HERBERT, University of Pennsylvania, Philadelphia, Pa.

Lane, Prof. Henry H., State University of Oklahoma, Norman, Okla.

Lee, Prof. F. S., 437 West 59th St., New York, City N. Y.

Lefevre, Prof. George, University of Missouri, Columbia, Mo.

Lewis, Prof. I. F., University of Virginia, Charlottesville, Va.

Lewis, Prof. W. H., Johns Hopkins University, Baltimore, Md.

Lillie, Prof. Frank R., University of Chicago, Chicago, Ill.

Lillie, Prof. R. S., Clark University, Worcester, Mass.

Linton, Prof. Edwin, Washington and Jefferson College, Washington, Pa.

Loeb, Prof. Jacques, Rockefeller Institute for Medical Research, New York City, N. Y.

Loeb, Dr. Leo, Washington University Medical School, St. Louis, Mo.

Lowther, Mrs. Florence DeL., Barnard College, Columbia University, New York City, N. Y.

Luscombe, Mr. W. O., Woods Hole, Mass.

Lyman, Prof. George R., Federal Horticultural Board, Washington, D. C.

Lynch, Miss Clara J., Smith College, Northampton, Mass.

Lund, Dr. E. J., University of Pennsylvania, Philadelphia, Pa.

McClendon, Dr. J. F., University of Minnesota, Minneapolis, Minn.

McClung, Prof. C. E., University of Pennsylvania, Philadelphia, Pa.

McGill, Dr. Caroline, Murray Hospital, Butte, Montana.

McGregor, Dr. J. H., Columbia University, New York City, N. Y.

McIndoo, Dr. N. E., Bureau of Entomology, Washington, D. C.

Mackenzie, Prof. Mary D., Carnegie Institute of Technology, Pittsburgh, Pa.

McMurrich, Prof. J. P., University of Toronto, Toronto, Canada.

Macklin, Dr. Charles C., Johns Hopkins University, Baltimore, Md.

Mall, Prof. F. P., Johns Hopkins University, Baltimore, Md.

Malone, Dr. E. F., University of Cincinnati, Cincinnati, Ohio.

Martin, Miss Bertha E., Wheaton College, Mass.

Marquette, Mr. William, Columbia University, New York City, N. Y.

Mathews, Prof. A. P., University of Chicago, Chicago, Ill.

Mayer, Dr. A. G., Maplewood, N. J.

Meigs, Dr. E. B., Dairy Division Experiment Station, Beltsville, Md.

Meltzer, Dr. S. J., 13 West 121st Street, New York City, N. Y.

Metcalf, Prof. M. M., 128 Forest St., Oberlin, Ohio.

Minor, Miss Marie L., Bryn Mawr College, Bryn Mawr, Pa.

Minoura, Mr. Tadachika, University of Chicago, Chicago, Ill.

Mitchell, Dr. Philip H., Brown University, Providence, R. I.

Moore, Prof. George T., Missouri Botanical Garden, St. Louis, Mo.

Moore, Mr. Carl H., University of Chicago, Chicago, Ill.

Moore, Prof. J. Percy, University of Pennsylvania, Philadelphia, Pa.

Morgan, Prof. H. A., Agricultural Experiment Station, Knoxville, Tenn.

Morgan, Dr. Anna H., Mount Holyoke College, South Hadley, Mass.

Morrill, Prof. A. D., Hamilton College, Clinton, N. Y.

Morrill, Dr. C. V., 338 East 26th Street, New York City, N. Y.

Morris, Miss Margaret, Yale University, New Haven, Conn.

Murbach, Dr. L., Central High School, Detroit, Mich.

Nachtrieb, Prof. Henry F., University of Minnesota, Minneapolis, Minn.

Neal, Prof. H. V., Tufts College, Tufts College, Mass.

Newman, Prof. H. H., University of Chicago, Chicago, Ill.

Nichols, Dr. M. Louise, 3221 Race St., Philadelphia, Pa.

Oliver, Mr. Wade W., Ohio-Miami Medical College, Cincinnati, Ohio.

Osburn, Prof. R. C., Connecticut College, New London, Conn.

Osterhout, Prof. W. J. V., Harvard University, Cambridge, Mass.

Packard, Dr. Charles, Columbia University, New York City, N. Y.

Packard, Dr. W. H., Bradley Polytechnic Institute, Peoria, Ill.

Painter, Mr. T. S., Yale University, New Haven, Conn.

Pappenheimer, Dr. A. M., Columbia University, Dept. Pathology, New York City, New York.

Parker, Prof. G. H., 16 Berkeley Street, Cambridge, Mass.

Paton, Dr. Stewart, Princeton University, Princeton, N. J.

Patten, Miss J. B., Elm Brook, South Natick, Mass.

Patten, Dr. William, Dartmouth College, Hanover, N. H.

Patterson, Prof. J. T., University of Texas, Austin, Texas.

Payne, Prof. F., University of Indiana, Bloomington, Ind.

Pearse, Prof. A. S., University of Wisconsin, Madison, Wis.

Phillips, Miss Ruth L., Western College, Oxford, Ohio.

Pike, Prof. Frank H., 437 West 59th Street, New York City, N. Y.

Pinney, Miss Mary E., Bryn Mawr College, Bryn Mawr, Pa.

Prentiss, Miss Henrietta, Normal College, New York City, N. Y.

Price, Dr. Weston A., Research Commission of the National Dental Association, Cleveland, Ohio.

Rankin, Prof. W. M., Princeton University, Princeton, N. J.

Rea, Dr. Paul M., Charleston Museum, Charleston, S. C.

Reighard, Prof. Jacob, University of Michigan, Ann Arbor, Mich.

Reinke, Mr. E. E., Vanderbilt University, Nashville, Tenn.

Rice, Prof. Edward L., Ohio Wesleyan University, Delaware, Ohio.

Richards, Dr. A., University of Texas, Austin, Texas.

Robbins, Mr. W. J., Cornell University, Ithaca, N. Y.

Robertson, Miss Alice, Wellesley College, Wellesley, Mass.

Robertson, Prof. W. R. B., University Club, Lawrence, Kansas.

Rogers, Prof. Charles G., Oberlin College, Oberlin, Ohio.

Rosenow, Dr. E. C., Mayo's Clinics, Rochester, Minn.

Ruddiman, Miss Marguerite, 441 Senator Street, Brooklyn, N. Y.

Sands, Miss Adelaide G., 348 N. Main St., Port Chester, N. Y.

Sands, Dr. Georgiana, 348 N Main St., Port Chester, N. Y.

Scott, Dr. Ernest L. Columbia University, New York City, N. Y.

Scott, Prof. G. G., College of the City of New York, New York City, N. Y.

Scott, Prof. John W., University of Wyoming, Laramie, Wyoming.

SHULL, DR. A. FRANKLIN, University of Michigan, Ann Arbor, Mich.

SHUMWAY, MR. WALDO, Amherst College, Amherst, Mass.

SMITH, DR. BERTRAM G., State Normal College, Ypsilanti, Mich.

SOLLMAN, DR. TORALD, Western Reserve University, Cleveland, Ohio.

SPAETH, DR. REYNOLD A., Yale University, New Haven, Conn.

SPAULDING, DR. E. G., Princeton University, Princeton, N. J.

SPENCER, DR. H. J., 8 West 16th St., New York City, N. Y.

STEWART, MISS MARY W., Barnard College, Columbia University, New York City, N. Y.

STOCKARD, PROF. C. R., Cornell Medical College, New York City, N. Y.

STREETER, DR. GEORGE L., Johns Hopkins Medical School, Baltimore, Md.

STRONG, DR. O. S., 437 West 59th St., New York City, N. Y.

STRONG, DR. R. M., Vanderbilt University, Nashville, Tenn.

STURTEVANT, MR. A. H., 528 West 123rd Street, New York City, N. Y.

TASHIRO, DR. SHIRO, University of Chicago, Chicago, Ill.

TAYLOR, MISS KATHERINE A., Cascade, Washington Co., Maryland.

TENNENT, PROF D. H., Bryn Mawr College, Bryn Mawr, Pa.

THOMAS, DR. ADRIAN, 2012 Hanover Ave., Richmond, Va.

THOMPSON, PROF. CAROLINE B., 195 Weston Road, Wellesley, Mass.

TINKHAM, MISS FLORENCE L., 71 Ingersoll Grove, Springfield, Mass.

TOMPKINS, MISS ELIZABETH M., 2015 Bedford Avenue, Brooklyn, N. Y.

TREADWELL, PROF. A. L., Vassar College, Poughkeepsie, N. Y.

TURNER, MR. C. L., Marquette Univ. School of Medicine, Milwaukee, Wis.

UHLENHUTH, DR. EDWARD, Rockefeller Institute for Medical Research, New York City, N. Y.

VAN CLEAVE, DR. H. J., University of Illinois, Urbana, Ill.

VAUGHAN, DR. T. W., U. S. Geological Survey, Washington, D. C.

WAITE, PROF. F. C., Western Reserve University Medical School, Cleveland, Ohio.

Waldron, Dr. F. R., 1701 Hill St., Ann Arbor, Mich.
Walker, Dr. George, Charles and Center Streets, Baltimore, Md.
Wallace, Prof. Louise B., Mount Holyoke College, South Hadley, Mass.
Warbasse, Mrs. J. P., 384 Washington Ave., Brooklyn, N. Y.
Ward, Prof. H. B., University of Illinois, Urbana, Ill.
Wardwell, Mr. E. H., Bristol, R. I.
Warren, Prof. Howard C., Princeton University, Princeton, N. J.
Wasteneys, Mr. Hardolph, Rockefeller Institute, New York City, N. Y.
Watson, Mr. Frank E., Hobart College, Geneva, N. Y.
Werber, Dr. E. I., Yale University, New Haven, Conn.
Weston, Mr. William H., Western Reserve University, Cleveland, Ohio.
Wheeler, Miss Isabel, Dana Hall, Wellesley, Mass.
Wheeler, Prof. W. M., Bussey Institution, Forest Hills, Mass.
Wherry, Dr. W. B., Cincinnati Hospital, Cincinnati, Ohio.
White, Miss E. Grace, Princeton University, Princeton, N. J.
Whitney, Dr. David D., Wesleyan University, Middletown, Conn.
Whiting, Mr. Phineas W., University of Pennsylvania, Philadelphia, Pa.
Wieman, Prof. H. L., University of Cincinnati, Cincinnati, Ohio.
Wilcox, Dr. Alice W., Fairbanks Museum of Natural History, St. Johnsbury, Vt.
Wildman, Dr. E., 4331 Osage Avenue, Philadelphia, Pa.
Williams, Dr. Anna W., 549 Riverside Drive, New York City, N. Y.
Wilson, Prof. H. V., University of North Carolina, Chapel Hill, N. C.
Woglom, Dr. William H., Columbia University, New York City, N. Y.
Woodruff, Prof. L. L., Yale University, New Haven, Conn.
Woodward, Miss Alvalyn E., Vassar College, Poughkeepsie, N. Y.
Young, Mr. D. B., Hartley Hall, Columbia University, New York City, N. Y.

AMITOSIS IN CELLS GROWING IN VITRO.

C. C. MACKLIN.

(*From the Department of Anatomy, Johns Hopkins University, and the Marine Biological Laboratory, Woods Hole, Mass.*)

(3 Plates, 27 Figures.)

Contents

Introduction 445
Method 446
Observations 448
  Nuclear division by amitosis 449
  Subsequent history of cell containing a directly divided nucleus 453
Discussion 455
Fragmentation 458
Vital stains 458
Summary 459
Literature cited 460
Explanation of Plates 462

Introduction.

During recent years the conviction among cytologists has become more and more strongly intrenched that the problem of amitosis can not be satisfactorily solved by investigations based alone upon the study of non-living tissue, but that its successful conquest must rely principally upon the correct interpretation of the succession of morphological and physiological changes revealed by prolonged observation of the living cell, under normal and artificially varied conditions. Yet until the advent of the tissue culture method, workers in biology might well have despaired of ever being able to attack the question in this way. It is fortunate, therefore, that the technique of tissue cultivation has been so perfected that even the minute structural details of the living cell may be readily observed, and that the inspection may be continued for hours at a time; fortunate, too, that configurations which may be interpreted as stages in the process of direct division, such as dumb-bell-shaped nuclei apparently undergoing constriction, and bipartite nuclei, are not infrequently found in tissue cultures, and, furthermore, that fixed and stained preparations, to assist in the interpretation of the appearances presented by the living cells, are easily made from such cultures.

These favorable circumstances plainly indicated the course to be followed in an attempt to gain some knowledge of direct cell division, and accordingly continuous observations of living cells, and supplemental studies of fixed and stained cells of the same type, were carried out.[1]

METHOD

The cells upon which observations were made were growing at a temperature of 39° to 40° Cent. from the tissue of embryo chicks from two to ten days old. Cultures were prepared by the method of Lewis and Lewis ('15), Locke solution being used as a medium. Activation of the growth was accomplished by the addition to the medium of a small quantity of autogenous embryo extract or bouillon. Thus the medium contained, besides the various salts, small quantities of dextrose (.25 to 1 per cent.) and protein. To offset the concentration due to evaporation during planting and in the moist chamber it was found to be of advantage to dilute the medium by adding 20 to 25 per cent. of freshly distilled sterile water. Heart tissue was most frequently used, and gave growths which were most serviceable for observation during the second twenty-four hour period.

By arranging the microscope within the incubator where the tissues were cultivated it was not necessary to expose them to a changed temperature during observation. A ray screen of copper sulphate solution was found to be advantageous when artificial light was used. Evaporation of the drop, with condensation about the walls of the moist chamber, was lessened by placing a small drop of distilled water in the cavity of the depressed slide, and by eliminating air currents from the vicinity of the culture.

Light seemed to have a deleterious effect upon the living cultures, so that lengthy continuous observation was not found to be practicable. Accordingly inspections were made as short as possible, and the light immediately turned off. The difficulties attending direct and prolonged observation of the living cell are not inconsiderable, for the eye must become accustomed to distinguish minute structures through the contrast afforded by varying grades of refractivity. At first only the most refractive bodies are discernible, standing out as bright points or lines, but gradually the less obvious structures, as mitochondria, and amœboid cell processes, come into view. Migration, in many of the cells, is quite active, and hence it is necessary to make observations frequently to prevent the cell from wandering away from the field of vision and becoming lost. The extreme sensitivity of the cell to light and heat, and to changes in osmotic pressure of the media from evaporation within the moist chamber, is responsible for the untimely termination of many observations, and this difficulty becomes the more important when it is realized that the study of a single cell must cover many hours to be complete.

---

[1] The procedure of studying amitosis by the tissue culture method was suggested by M. R. and W. H. Lewis, and I desire to record my appreciation of their kind assistance and the use of their large collection of fixed and stained cultures, which was utilized in the investigation. To Prof. F. R. Lillie I also am indebted for his courtesy in placing a room at the Marine Biological Laboratory at my disposal, where some of the work was carried on.

It was at first planned to select a single living normal cell and observe it at frequent intervals over a long period in the hope that eventually a cell would be found which would divide by amitosis. This course, however, did not prove to be practicable on account of the infrequency with which amitosis, even of the nucleus alone, is met with. In a series of 20 fixed and stained growths from the heart of the embryo chick, in which there was a total of 41,725 cells,[1] only 50 constricted nuclei, which could be regarded as directly dividing, were found (a ratio of 1 to 835), and thus the chances of the occurrence of amitotic nuclear division in a cell selected at random were but little better than one tenth of one per cent. To avoid loss of time, therefore, the plan was adopted of selecting a cell in which the amitotic process appeared to have already commenced, viz., in which the nucleus showed elongation and equatorial constriction, and observing in detail the subsequent changes which it underwent.

---

[1] The method adopted in making counts was as follows: 20 good preparations from cultures of chick heart of various ages and stages of growth which had been fixed and stained were selected. A small square was ruled with a diamond upon a piece of glass after the method of Isaacs ('15), and this ruled glass was inserted in the ocular so that a definitely outlined field was marked off upon the tissue culture preparation on the stage. By manipulating the mechanical stage successive fields could easily be brought into view, and the cells contained in them counted; in this way all the cells in the entire new growth were counted except imperfect cells and those near the original piece, which were several layers deep and were indistinct.

Such a cell presents an appearance of the following general type. The nucleus is somewhat lengthened, and, in the zone equidistant from its poles there is to be seen, on one or both sides, an indentation. In this concavity is situated characteristically a body, the centrosphere (fig. 1, *c*) whose refractivity is somewhat greater than that of the surrounding cytoplasm. Its outline is indistinct, but the edge seems to be irregular with short toothlike processes. These change their shape slowly, and give the impression of being pushed out and drawn in very gradually. They are intimately related to definite refractive bodies—the mitochondria—which, often rodlike and sometimes threadlike in form, radiate from the periphery of the centrosphere. Indeed the movements of the latter (described by Lewis and Lewis '15) may be responsible for the apparent movement in the periphery of the centrosphere.

Preparations fixed with osmic acid vapor and stained with Heidenhain's iron hematoxylin (which was the method generally employed) disclosed a minute granular body, generally paired, within the centrosphere, which is recognized as the centrosome or centriole (Fig. 24).

The position of the centrosphere within the nuclear concavity or cleft has been noted by various authors, including Maximow ('08), who found the centriole-pair thus situated in cells, the nuclei of which appeared to be dividing directly, in the mesenchyme of the embryo rabbit. In the cells of tissue cultures examined the centrosphere was absent from the cleft in only two per cent. of cases, and in these exceptions it may have been originally situated as in the others.

Observations.

A number of extended observations were made upon living cells containing such elongated and constricted nuclei, and, as a general rule, the nucleus rounded out again, assuming the usual form. Thus it was demonstrated that constriction alone does not indicate that the nucleus will divide directly. Finally, however, a cell was found in which the nucleus divided directly while being watched, and the following paragraph, extracted from the protocol written at the time, is a brief description of the process as observed. The drawings (Plate I.) were sketched free-hand from observation at fifteen-minute intervals, and afterward retouched by reference to fixed and stained cells of similar morphology.

8.45 P.M. A cell (Fig. 1), growing from a 57-hour culture of 5-day chick heart, presented an elongated nucleus with a concavity upon one side. The outline of this concavity was indistinct on account of the fact that the centrosphere (*c*) was situated very close to it.

9.00 P.M. (Fig. 2). The nucleus is now straight and the indentation is almost obliterated.

9.15 P.M. (Fig. 3). The general outline of the cell has changed, and this has been followed by change in shape of the nucleus. At first there were two nucleoli within the nucleus, the lowermost being paired, but at 9.30 (Fig. 4) the latter appears as a dumb-bell-shaped body. The nucleus is now rounded.

9.45 P.M. (Fig. 5). The nucleus is still rounded, and the nucleolar substance consists of two masses close together.

10.00 P.M. (Fig. 6). The nucleus has become elongated again. There is no apparent cleft, but the left side, against which the centrosphere is resting is not so distinct as the right. The central nucleolus now appears as a single structure and some refractive substance, resembling another nucleolus, is seen in the lowermost pole of the nucleus.

During the next two fifteen-minute intervals (Figs. 7 and 8) a shortening of the nucleus occurred, a shallow concavity to the left being noted. This concavity is deeper at 10.45 (Fig. 9) its outlines being somewhat indistinct and the nucleus has increased in length; across the middle of the nucleus is a refractive line.

11.00 P.M. (Fig. 10). The concavity is seen with difficulty, and the line across the nucleus persists. Fifteen minutes after this (Fig. 11) the elongated nucleus shows an indentation upon the right side, opposite the one upon the left, and it appears to be undergoing constriction into two parts of equal size. Between the two nuclear portions the refractive line is seen as before, and, from the appearance shown in cells fixed and stained with iron hematoxylin (Fig. 24) this is evidently a strand of mitochondria.

11.30 P.M. (Fig. 12). The nuclear sacs are apparently quite separate, and between them is the strand of mitochondria, and also part of the centrosphere, this body, still undivided, having retained its position with reference to the nuclear cleft.

Judging from the behavior of this and other elongated, bent and dumb-bell shaped nuclei it would seem that the nucleus may return to its original rounded form provided the constriction has not gone too far, but, if the degree of constriction passes a certain critical point the nuclear sacs become completely separated.

Furthermore, after the critical point is passed the division occurs very rapidly.

The study of fixed and stained specimens served to throw considerable light upon the process of direct division, for, by searching the field, transitional forms were encountered, suggesting stages in the history of the living nucleus just described. In the terminal stage of direct nuclear division, such as that shown in Fig. 24, mitochondria were found characteristically lying across the slender strand of nuclear membrane which was all that remained of the connection between the two portions of the nucleus, and the centrosphere (which is undivided in these transitional forms, and also in the end product of nuclear amitosis, the binucleate cells) is situated in the cleft dividing these parts.

This process involves a more or less equal mass division of the nucleus without chromosome formation. It results in the production of a binucleate cell, and, if the process is repeated, of a trinucleate or multinucleate cell. Direct nuclear division was not observed beyond the bipartite stage, but it seems rational to suppose that (excluding the foreign body giant cells of Lambert (1912 *a* and *b*) the giant cells of tissue cultures are formed from the successive direct splitting of the nuclear fragments which become larger by normal growth. With this latter there is apparently associated an increase in the cytoplasm also.

It was not practicable to make a complete study of amitosis upon the same cell, and the latter stages of this process, succeeding direct division of the nucleus, were studied by selecting living binucleate cells and making prolonged observations upon them. A typical case, from a 24-hour culture of 8-day chick heart, is given as follows:

11.50 A.M. The cell, which was of the characteristic connective tissue type, showed two separate nuclear sacs, whose adjacent surfaces were in close contact. One sac contained three nucleolar fragments, the other one. Fat granules were fairly abundant, and were principally congregated at the nuclear poles. Mitochondria, long and threadlike, stretched between these granules and, where the latter were abundant, the strands of mitochondria tended to arrange them in rows. Mitochondria also radiated from the single centrosphere, situated opposite the area of contact of the two nuclear sacs. The triangularly shaped cell body was connected with adjacent cells by three main processes.

12.20 P.M. A narrow interval can be seen between the nuclear parts, showing that the latter have moved apart and are quite separate. Their position also has changed. Variation in the nucleoli is noted, there being now three in one nuclear sac and four in the other. The outline of the cell body is now quadrilateral, and this shifting of form has perhaps accounted for the rearrangement in relative position and relationship of the nuclear parts. These, at 2.30, were again in contact, but subsequently repeated the process of moving apart and coming together three times during the observation, which ended at 11.15 p.m. During this time (11½ hours) the cell was observed continuously, and underwent constant minor changes, such as that of the outline, shifting about of cytoplasmic structures, and breaking up, recombination and variation in size and shape of the nucleolar fragments. After almost twelve hours the cytoplasm showed no indication of dividing.

These observations brought out the fact that what might be mistaken for a single nucleus divided by a membrane across its equator is really two nuclear sacs pressed close together, the equal tension in the two bodies resulting in a flat membrane between them, made up of the surfaces of contact. Child (1911) describes a type of amitosis which is characterized apparently by the growth through the nucleus of a membrane or plate, the subsequent splitting of which leads to the production of two nuclear sacs quite separate one from the other. Nuclear fission of this type was not found, and appearances suggesting a process of this kind probably result from the close relationship of separate nuclear parts, similar to the condition found in the cells of tissue cultures. Partitions within the nucleus are also simulated, in these flattened cells, by long nucleoli, mitochondria or folds in the nuclear membrane.

These observations also illustrate the characteristic behavior of the nucleolar bodies, which undergo constant changes in size, shape, number and position. These bodies stain well with gentian violet when applied to the living culture. They appear as dark masses after iron hematoxylin, but if differentiation with iron alum is carried too far what was before a homogeneous mass becomes a collection of granules (Fig. 27). The nucleolus appears to be a concentrated gel of varying density, the granules representing the denser areas.

Similar observations upon other living cells were carried out, and in no case did any direct fission of the cytoplasm occur; this finding was supported by the study of fixed preparations. On the contrary the history of these binucleate cells was the same as that of mononucleate cells of the same type. It was even found that mitosis occurred in these cells, for, during the observation of a binucleate cell in a young culture the good fortune was encountered of witnessing this entire process in it. A graphic register of the successive changes is afforded by the drawings (Plate II.) which were made with the aid of a camera lucida at the times stated. The following is extracted from the protocol written at the time of examination:

11.55 A.M. A typical connective tissue binucleate cell (Fig. 13) from a 19-hour culture of 7-day chick heart was selected for observation. The two approximately equal, sharply outlined nuclear sacs are in close contact, causing a flattening of the apposed surfaces. Each nucleus contains a single, somewhat irregular nucleolus. The cell is long and narrow, and the long axis of the nucleus is parallel with that of the cell in which it is contained. A single centrosphere (*c*) is found opposite the area of contact of the two nuclear parts. Numerous fat globules and mitochondria, the latter showing characteristic movements, are seen.

12.40 P.M. (Fig. 14). The nucleus is still double, and the principal change noted is the appearance of an additional nucleolus in the lower nuclear part.

1.20 P.M. (Fig. 15). The two parts of the nucleus are distinct. Only one nucleolus is now seen in each nuclear sac.

1.50 P.M. (Fig. 16). The cell outline has become modified, the cell body being shorter, and the long axis of the nucleus has changed so that it is now almost at right angles to its former direction. The nuclear surfaces are in close contact and the upper end of the membrane formed by their approximation is indefinite in outline.

3.05 P.M. (Fig. 17). The cell body has become more fusiform, and the long axis of the nucleus has rotated through 90°. The double membrane (formed by the areas of nuclear wall in contact) dividing the nuclear portions cannot be made out except at the left side, and there indistinctly. An indefinite, refractive substance, granular in character, is scattered along the line where this membrane had been; the nucleoli are indefinite, and the uppermost one has been joined by an additional, very small, mass of the same character. The upper part of the nuclear membrane is not so distinct as heretofore.

It would appear that the change which has occurred in the part of the nuclear membrane dividing the two nuclear sacs is a part of the general change affecting the entire nuclear wall and leading to its gradual disappearance, and the process is similar to that which occurs in the early stages of mitosis.

5.05 P.M. (Fig. 18). The refractive material in the zone formerly separating the two nuclear sacs is more prominent than before; it seems to be chromatin. The nuclear membrane is faintly marked.

Half an hour later this cell is seen to become gradually smaller, and to draw in its processes. At the same time the nuclear parts become smaller. The nucleoli also undergo diminution in size. Finally the cell takes a rounded and thickened form—6.00 P.M. (Fig. 19)—and is much more refractive than the cells surrounding it: in fact it resembles a cell in the prophase of mitosis. The fat globules and mitochondria assume a wreath-like appearance about the central clear space, in which the nucleoli soon disappear. Though the main mass of the cell is almost circular there are narrow processes attached to each pole. The cell remains apparently unchanged for some time, though undoubtedly important readjustments are going on within it.

6.50 P.M. (Fig. 20). The cell is even smaller than before, and more rounded. In the clear area within it is a refractive bar, which proves to be the equatorial plate of chromosomes. Individual chromosomes cannot be distinguished, so that it is impossible to count them, but their ends may be seen, as they project towards the poles of the spindle. The chromosomes show a slow, oscillatory type of movement, slight in extent. The spindle is represented by a fairly clear area, shaped like two cones base to base, at the extremities of which the centrosomes are situated. Astral rays cannot be seen. Mitochondria and fat globules encase this central area containing the spindle like a shell. This becomes evident by focusing up and down. Though the uppermost part of the spherical cell is on a level with the flat resting cells, which are to be found about it, the lowermost part is much below this, due to the fact that the cell is thickened, and projects into the medium.

7.05 P.M. (Fig. 21). The cell has suddenly become elongated and constricted at the equator; the plate of chromosomes has evidently split, and the anaphase of mitosis is being witnessed. The constriction about the middle of the cell can be seen to be increasing, causing streaming of globules toward its extremities. At the same time small, bubble-like processes emerge from the borders of the cell, seeming to be forced out by the internal pressure of the cell body. Into these protuberances granules flow but later return into the main mass of the cytoplasm. These processes soon become flattened and extended, forming pseudopodia possessed of hyaline borders with amœboid movement. The individual chromosomes of the two masses in the expanded extremities gradually lose their distinctness and become dispersed.

7.25 P.M. (Fig. 22). The constriction is more marked and the cells are almost entirely separated. They are also becoming flattened out. In the upper daughter cell a clear space for the nucleus is appearing. The constricted zone is somewhat more highly refractive than the surrounding tissue and resembles a short thread. This probably contains part of the remains of the spindle. Nuclear details are not yet visible.

As the cell is watched nucleoli become manifest, at 7.35 P.M. two of these being seen in each daughter nucleus in a clear space, surrounded by a distinct nuclear membrane.

8.00 P.M. (Fig. 23). The daughter cells are now almost of normal size. Each nucleus has a concave side, as is usual, and in each concavity is the new centrosphere, from which the mitochondria radiate. Two nucleoli appear in each nucleus. The cells are spread out and flattened, and the fat granules are disposed as in the ordinary cell.

The entire observation covered eight hours. The more active part of mitotic division occupied about two hours, but if the initial nuclear changes be included the duration of mitosis is much longer.

The sequence of changes followed in the above cell are in almost all respects similar to those of mitosis many times observed in the mononucleate cell. The only difference is that in the cell described there were to begin with two separate nuclear sacs instead of one. From the presence, in fixed preparations, of bipartite nuclei each portion of which is in a condition of spireme, and the absence, from such preparations, of bipartite nuclei in which one portion only contains a skein, it is gathered that the prophase in these cells is characterized by the spireme appearing coincidently in the separate nuclear sacs (Fig. 25); later there is formed from the double spireme a single equatorial plate of chromosomes. Though these could not be counted there is no reason for believing that their number was more than that normal for the mononucleate cell.

Not only has mitosis been demonstrated by observation, both on living and fixed preparations, to occur in binucleate cells, but it has been found that it occurs relatively as frequently in these cells as in those with a unipartite nucleus. By making cell counts in the aforementioned 20 preparations from chick heart 375 binucleate cells were found in a total cell count of 41,725. Thus binucleate cells made up a percentage of the total of 0.9, or a ratio of 1 in 111. Among these binucleate cells 2 were found which were in the prophase of mitosis,[1] a percentage of 0.53. Of the mononucleate cells, which numbered 41,106, 47 were in the prophase, or 0.114 per cent. By comparison of these ratios it is found that mitosis occurs 4.65 times as frequently among the binucleate as among the mononucleate cells; allowing for the limited scope of the observation it seems reasonable to conclude that mitosis is as frequent a phase of the life of binucleate cells as of mononucleate, and it would seem that this is their normal method of proliferation. If, in addition, they be considered as dividing by direct fission (for which there is no evidence either from living or fixed material) they would then be possessed of an ability to multiply in excess of that of the mononucleate cells, and there is no reason for supposing this to be the case.

No evidence was brought to light that the separate parts of the bipartite or multipartite nucleus ever combine except during mitosis.

Another interesting observation was made in a fixed preparation, viz., that the early changes in the chromatin which presage the onset of mitosis (*i. e.*, the clumping of the chromatin and its segregation into short rodlike masses and finally into a spireme) can take place in a nucleus which is undergoing constriction. Thus it appears that mitosis can proceed as usual in nuclei partially divided or wholly divided by the amitotic process.

---

[1] Prophases alone were counted, in estimating cells in mitosis, since in this stage alone is it possible to distinguish the bipartite from the monopartite nucleus, on account of nuclear fusion in stages later than this in the case of mitosis in the binucleate cell.

Discussion.

The direct division of the nucleus is associated with certain changes of the cell as a whole. Elongation of the nucleus seems to be a prerequisite, and this apparently is secondary to a lengthening and narrowing of the cell body occasioned by the pull of its processes. It has been pointed out that the centrosphere is situated characteristically in a concavity at one side of the nucleus, and, when the nucleus lengthens, this body sinks deeper into its side; at the same time, judging from fixed preparations, and also from the appearance of the living and dividing nucleus, mitochondria come to lie across this narrow nuclear isthmus. These bodies, the centrosphere and associated mitochondria, seem to play a part in the fission of the nucleus. The exact manner of their action is not clear, but it may be that streaming of the nucleoplasm away from the equator of the nucleus follows upon the mechanical irritation of the nuclear membrane by their movements, or possibly upon local alteration of surface tension from their chemical change. Certainly the position which these bodies take with reference to the constricted nucleus points to their participation in the process of fission.

The centrosphere does not divide, nor does it encircle the nucleus as in the form of division described by Meves ('91). The nuclear membrane remains intact and nothing resembling an amphiaster is formed.

Some theories of amitosis have attempted to place the responsibility for the initiation of the divisional stimulus upon the nucleolus, and it has been found by some investigators that in certain nuclei the nucleolus is the first body to become divided. Such a function of the nucleolus does not obtain, however, in the nuclei studied, for, although in some cases there was a division of the nucleolus into two parts, one of which became alloted to each separate nuclear part, yet, sometimes the nucleolus did not divide, and one of the nuclear fragments was without visible nucleolar material (Fig. 24). Occasionally two fragments would be found in one nuclear part while the other had none; again it was very frequently found—indeed it was the rule—that a nucleus would have two separate nucleolar fragments without any nuclear division. In short not only was the division of the nucleolus in most cases not followed by nuclear fission, but the latter took place in many cases without division of the nucleolus.

Neither before nor after nuclear fission was there discovered an instance of differential staining of the two halves of a nucleus about to be divided, or already divided, as shown by Child ('07) so that there was no evidence of this kind to support the belief of Child that there may be a physiological independence of the nuclear areas even before they become amitotically divided, manifested by a variation in the staining of the two nuclear halves. The study of living nuclei, too, divulged nothing in favor of this hypothesis.

The result of this process of nuclear splitting was the formation of a cell containing one or more separate nuclei of about equal size, and each of about the same size as the nuclei of mononucleate cells. After nuclear fission the separated nuclear elements manifested the power of growth, and seemed to have metabolic independence. The cell protoplasm also of these cells shows an ability to increase in bulk. This is especially evident in giant cells which can thus become quite large.

It is believed, furthermore, that binucleate cells and giant cells in tissue cultures (except foreign body giant cells which arise by fusion of previously separate cells) do not arise in any other manner than that above outlined, for there is no other adequate explanation of their origin. A careful examination of living and fixed material does not reveal any evidence of fusion of cytoplasm without fusion of the nuclei, so that there are no grounds for admitting this as a possible theory of formation. Although many of the binucleate cells undoubtedly do migrate as such from the original piece, where they are doubtless formed in the same manner as they are in the new growth, yet an increase of over 100 per cent. in their number in the growth of the second day as compared with that of the first (as ascertained by making careful counts of the 20 heart specimens aforementioned) can hardly be explained by the assumption of an increased emigration of these forms during the second day; it is more probable that some of these bipartite nuclei have originated in the new growth, and the observation of this process here confirms this conclusion.

Mitosis, too, cannot account for the formation of these bi- and multipartite nuclei, for in all the cases of mitosis followed through in the living condition the end result has always been two daughter cells, quite separate except for a narrow connecting process, and each containing a single centrosphere. The only theories remaining for consideration are those of nuclear origin *de novo*, or from chromidial extrusions, and these suppositions are too improbable to discuss here. No other process than that of nuclear amitosis, therefore, can account for the production of bi- and multinucleate cells in the cultures examined.

Though the amitotically-divided parts of the nucleus seem to possess metabolic independence, as noted, they do not appear to have reproductive independence, for the reason that they are never dissociated one from the other to become the nuclei of separate cells. Furthermore they show only one type of cell division, viz., mitosis, in which the process begins coincidently in the two nuclear parts, manifested by the simultaneous appearance in each part of a similar spireme. Although in amitosis there is a mass division of the nuclear material there is no meristic division, and it appears that before a cell containing an amitotically divided nucleus can divide it is necessary that the separated chromatin moieties should recombine. This was done in the specimen examined during life, for the combined product of the two nuclear sacs formed a single equatorial plate of chromosomes. Such a type of amitosis, therefore, is not incompatible with the chromosome hypothesis.

Spiremes in bipartite nuclei, and in dumb-bell-shaped nuclei evidently undergoing amitosis, are not confined to the cells of tissue cultures, for Maximow ('08) has described them in the normally-developing mesenchyme cells of the embryo rabbit, and this author refers to similar cell configurations which Karpow ('04) describes in the leucocytes of urodele amphibia.

FRAGMENTATION.

A note may here be made regarding a pathological change which nuclei subjected to unfavorable conditions undergo, viz., fragmentation. This change consists in a breaking up and degeneration of the nuclei. In cells which had grown for six days in unchanged media, in which food and oxygen had become depleted and metabolic products had accumulated (Fig. 26) and also in cells growing in a medium to which a small amount of ethyl alcohol had been added (Fig. 27), this form of degeneration was seen. Multilobulation of the nucleus appeared to antecede the actual breaking away of the parts. These latter were of different shapes and sizes, often did not contain a nucleolus, and showed no power of growth. The cytoplasm enveloping them did not divide and apparently was incapable of increasing.

In preparations containing forms of this kind no mitoses were seen, and the phenomenon seemed to be quite different from nuclear amitosis which occurred only in healthy cells. It is believed, moreover, that the process of fragmentation has been confused with that of amitosis, and it is possible that it is this confusion which has accounted for certain well-known views which regard amitosis as an evidence of degeneration.

VITAL STAINS.

Finally, a word as to certain so-called "vital stains." It was hoped that gentian violet would prove to be of value in rendering visible the *minutiæ* of the living cell during its vital changes, since the results following the use of this dye recorded by Churchman and Russell ('14) and Russell ('14) with cultures of frog tissue were so encouraging. Unfortunately the dye proved toxic to the tissues used in a dilution of 1 in 200,000, and, though the nucleoli, nuclear membranes, certain granules and the cell borders were brought into sharp relief this staining was always accompanied by cessation of vital phenomena, and the cells speedily went into degeneration.

Janus green, in a dilution of 1 in 80,000, was also toxic, and, while it stained mitochondria specifically, yet these bodies soon became granular and lost their characteristic appearance. Hence neither of these dyes could be spoken of as acting "*intravitam*," and they were of value only in obtaining rapidly information as to the obscure structure of the cell; the cell, however, was thus sacrificed.

SUMMARY.

The observations above described and the interpretations thereof may be briefly summarized as follows:

Amitosis was found to involve only the nucleus and was not a method of cell proliferation. It occurred in normal cells and was characterized by a separation of the nucleus into one or more parts which possessed no reproductive independence.

The process of nuclear amitosis consisted in a unilateral or bilateral constriction, manifested by a narrowing of the nucleus in the region of its equator, and a streaming of its contents toward the poles, with final separation of the two nuclear portions. This phenomenon seemed to be associated with the action of the mitochondria and centrosphere upon an elongated nucleus. There was no amphiaster or spireme formation and no centrosome fission. Division of the nucleolus was not an essential. Repetition of this process leads to the formation of a giant cell.

Not all nuclei which show elongation and constriction divide by direct fission, but many return to their usual rounded or oval form. When, however, the constriction has passed a critical point the division goes on to completion, and this final stage is rapid.

Cells containing nuclei in process of, or the result of, amitosis divide by mitosis. Mitosis in binucleate cells, which are the product of nuclear amitosis, is characterized by the simultaneous appearance in the nuclear parts of a spireme, from which a single equatorial plate of chromosomes is formed. Furthermore, binucleate cells divide as frequently by mitosis as do mononucleate cells, and this was the only form of division found to occur in them.

Since the parts of an amitotically divided nucleus do not become separated as reproductive units but divide only by mitosis, in which the chromatin in the parts is recombined, there is nothing in nuclear amitosis opposed to the chromosome hypothesis.

The type of amitosis in which the nucleus is split by the growth through it from one side to the other of a membrane was not found. Nuclear figures simulating this proved to be caused by the close apposition of separate nuclear sacs, or by nucleoli, mitochondria or folds of the nuclear membrane.

The dyes, janus green and gentian violet, were toxic and their presence in the cell was incompatible with its continued life. They were, however, of service in quickly studying structural details which were not discernible in the living state.

Nuclear fragmentation, which differs in many ways from nuclear amitosis, is a pathological condition, and occurs in degenerating cultures.

It is believed that the facts brought to light through the tissue culture method may be applied to the interpretation of the phenomena of normally developing cells.

LITERATURE CITED.

Child, C. M.

'07 Studies on the Relation between Amitosis and Mitosis. IV. Nuclear Division in the Somatic Structures of the Proglottids of Moniezia. V. General Discussion and Conclusions Concerning Amitosis and Mitosis in Moniezia. BIOL. BULL., Woods Holl, XIII., 165–184.

'11 The Method of Cell-Division in Moniezia. BIOL. BULL., Woods Hole, XXI., 280–296.

Churchman, J. W., and D. G. Russell.

'14 The Effect of Gentian Violet on Protozoa and on Growing Adult Tissue. Proc. Soc. Exper. Biol. and Med., N. Y., XI., 120–124.

Isaacs, R.

'15 A Mechanical Device to Simplify Drawing with the Microscope. Anatomical Record, Phila., Vol. 9, 711–713.

Karpow, W.

'04 (Untersuchungen über direkte Zellteilung.) Inaug.-Diss. Moskau, 1904. *Refer. in* Jahresber. ü. d. Fortschr. d. Anat. (Schwalbe), Jena, n.F., X., 42.

Lambert, R. A.

'12a Variations in the Character of Growth in Tissue Cultures. Anat. Rec. Phila., VI., 91–108.

'12b The Production of Foreign Body Giant Cells in Vitro. Jour. Exper. Med., Lancaster, Pa., XV., 510–515.

Lewis, M. R., and W. H. Lewis.

'15 Mitochondria (and Other Cytoplasmic Structures) in Tissue Cultures. Amer. Jour. Anat., Phila., XVII., 339–401.

Maximow, A.

'08 Ueber Amitose in den embryonalen Geweben bei Säugetieren. Anat. Anz., Jena, XXXIII., 89–98.

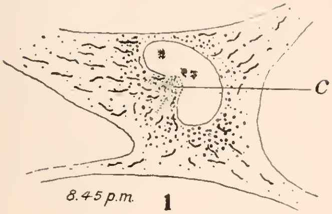
Meves, Fr.
'91 Ueber amitotische Kernteilung in den Spermatogonien des Salamanders und Verhalten der Attraktionssphäre bei derselben. Anat. Anz., Jena, VI., 626–639.

Russell, D. G.
'14 The Effect of Gentian Violet on Protozoa and on Tissues Growing *in vitro*, with Especial Reference to the Nucleus. Jour. Exper. Med., Lancaster, Pa., XX., 545–553.

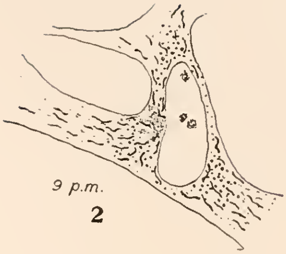
EXPLANATION OF PLATES.

Plate I.

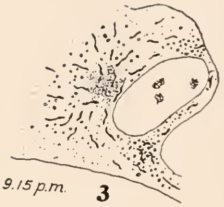
Figs. 1 to 12. Successive stages covering a period of 2¾ hours in the life history of a connective tissue cell from a 57-hour culture of 5-day chick heart, growing in Locke solution (0.5 per cent. dextrose with extract of chick embryo). The nucleus finally divides by direct fission. Small circles represent fat globules and short threads mitochondria. *c*, Fig. 1, points to the centrosphere. Free-hand drawings × about 900. (Description in text.)



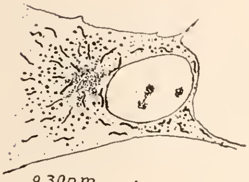
8.45 p.m. **1**



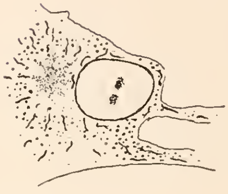
9 p.m. **2**



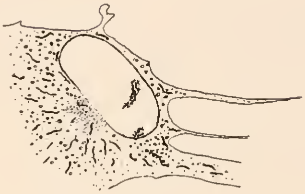
9.15 p.m. **3**



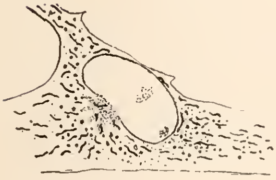
9.30 p.m. **4**



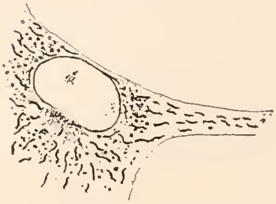
9.45 p.m. **5**



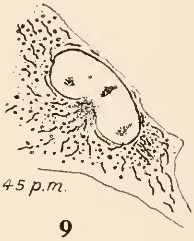
10 p.m. **6**



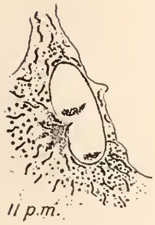
10.15 p.m. **7**



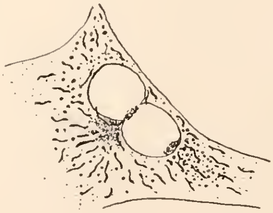
10.30 p.m. **8**



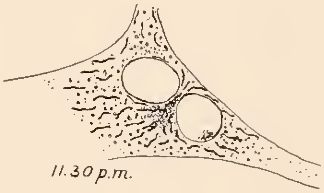
10.45 p.m. **9**



11 p.m. **10**



11.15 p.m. **11**

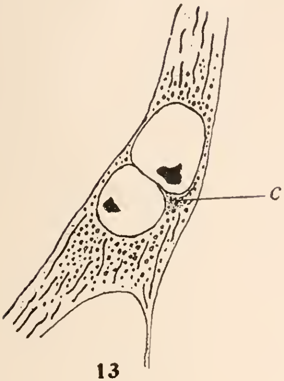


11.30 p.m. **12**

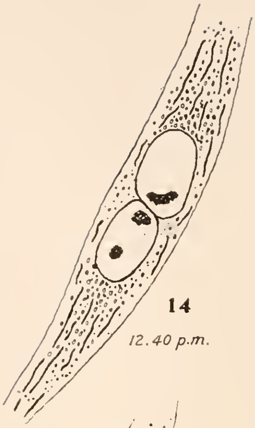
C. C. MACKLIN.

PLATE II.

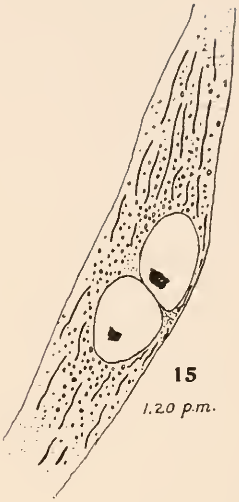
FIGS. 13 TO 23. Graphic record of the process of mitosis in a living binucleate cell, from a 19-hour culture of 7-day chick heart, growing in Locke solution (1 per cent. dextrose with extract of chick embryo). *c*, Fig. 13, points to the centrosphere. Camera lucida drawings. × 1,333. (Description in text.)



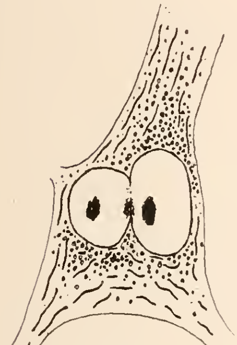
13
11.55 a.m.



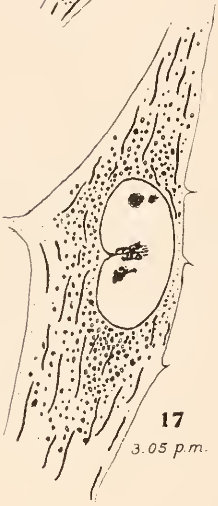
14
12.40 p.m.



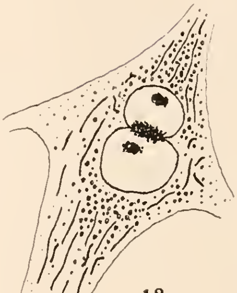
15
1.20 p.m.



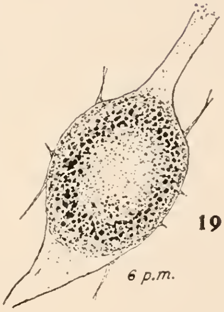
1.50 p.m.
16



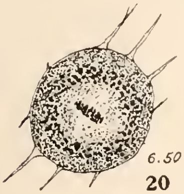
17
3.05 p.m.



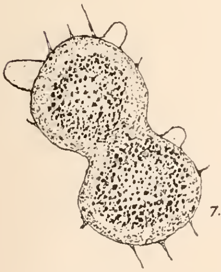
18
5.05 p.m.



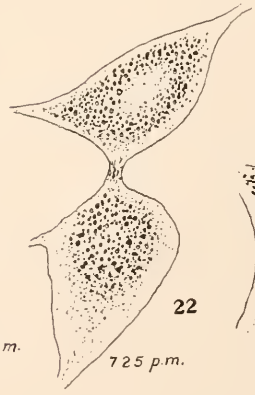
19
6 p.m.



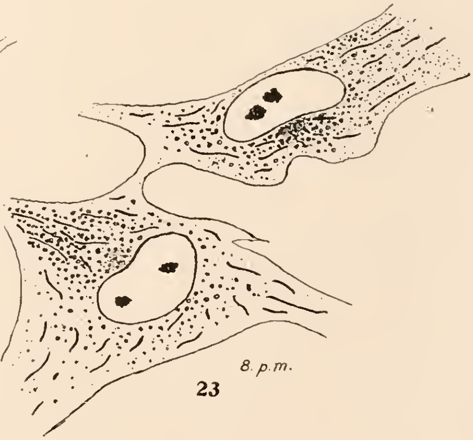
6.50 p.m.
20



21
7.05 p.m.



22
7 25 p.m.



8. p.m.
23

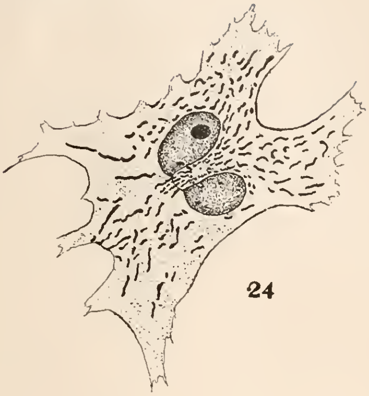
PLATE III.

FIG. 24. Direct nuclear division in a connective-tissue cell, final stage. Centrosphere between the nuclear parts. Across the slender filament joining these is a strand of mitochondria. Nucleolus has not divided. Camera lucida drawing from Preparation No. 2, from 5-day culture of 7-day chick heart in Locke solution (1 per cent. dextrose); osmic acid vapor and iron hematoxylin. × 915.

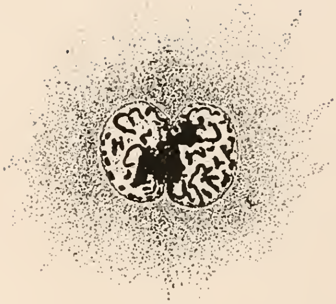
FIG. 25. Spireme in a bipartite nucleus. Prophase of mitosis. Nuclear membrane and nucleoli are disappearing. Camera lucida drawing from Prep. No. 14, 9–1–15 (Lewis collection). Heart from 6-day chick grown in Locke (0.5 per cent. dextrose) with a little yolk; fixed on third day of growth in Zenker; stained with iron hematoxylin (this culture was originally stained with Mallory's connective tissue stain). On account of the method of fixation the cytoplasmic details are not shown. × 915.

FIG. 26. Nuclei showing fragmentation. Camera lucida drawings from Prep. No. 23, 12–1–15 (Lewis collection). 5-day chick stomach in Locke (0.5 per cent. dextrose). Zenker; Mallory connective tissue stain. Culture grown for 6 days in the same media. × 1,012.

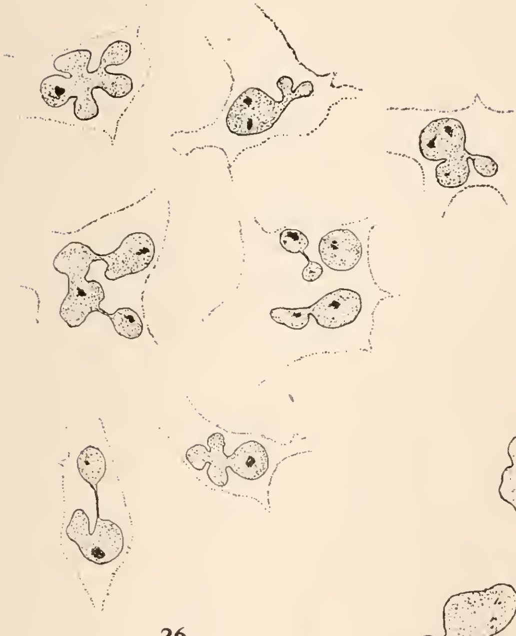
FIG. 27. Nuclei showing fragmentation. Camera lucida drawings from Prep. No. 23, 24–11–14 (Lewis collection). 6-day chick stomach in Locke (1 per cent. dextrose) to which ethyl alcohol had been added to make approximately 1 per cent. 3-day culture. Osmic acid vapor and iron hematoxylin. × 1,500.



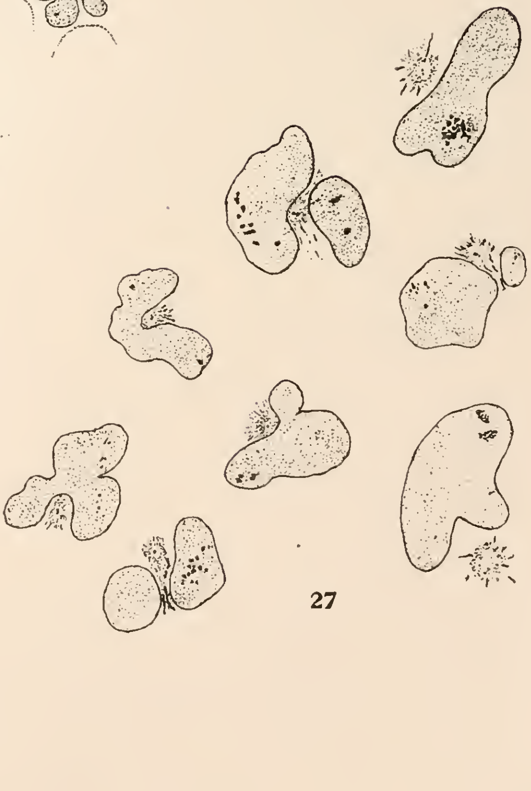
24



25



26



27

C. C. MACKLIN.

MBL/WHOI LIBRARY
WH 17K2 -

10931